term studies that rutin and quercetin, compounds of known composition and purity, have the therapeutic property of restoring to normal spontaneously increased capillary fragility and permeability in humans. That is, they, and likely other related flavonoids, possess definite and valuable drug action. We wish to especially emphasize the fact that the mechanism, or mechanisms, of action of these flavonoids as therapeutic agents, and their role in animal physiology, is not fully understood. Evidence does exist, however, that in addition to their specific action on the capillaries, these compounds exert a sparing action on epinephrine, vitamin C, and possibly other vitamins and hormones; and that they may alleviate conditions or have prophylactic action in cases where capillary fault is not necessarily involved. Much research must be done before the role of flavonoids in therapy, in animal nutrition, or even in plant metabolism, is understood.

Following the report that rutin corrects capillary fault in man, clinical work on the drug's application expanded rapidly. To meet the increasing demands for supplies of rutin, a number of pharmaceutical manufacturers were encouraged to start production in the summer of 1945. Since then its use in medicine has become widespread and it has been admitted to the ninth edition of the National Formulary.

A number of factors have contributed to the progressive growth and popularity of the use of rutin as a therapeutic agent. The early suggestion that rutin might decrease the incidence of hemorrhage, supported by data demonstrating its effectiveness in correcting capillary fault, seems to have strongly appealed to both physicians and the lay public. In this connection, previous publicity concerning the existence of anti-hemorrhagic factors which might prove to be prophylactics for such type of vascular accidents as retinal hemorrhage, coronary occlusion and apoplexy created a receptive atmosphere. Favorable to its acceptance, also, were the facts that it is a definite chemical compound permitting precise dosage; it is completely nontoxic with no side effects; it is easily purified and manufactured from cheap and readily available source materials.

Finally, it is our belief that the use of rutin and other flavonoids has steadily increased chiefly because of their real therapeutic value. The time factor required for their thorough evaluation in human medicine, especially in the type of case chosen for this purpose (hypertensives with spontaneous capillary fault) has delayed this comprehensive evaluation. Now, following 12 years of research, it appears to us that a definite evaluation is at last possible; to provide this is the main objective of our book.

The Chemistry of Rutin and the Technology of its Manufacture

Introduction and History

Rutin was discovered by Weiss[774] in 1842 in the leaves of a rue (*Ruta graveolens*). Subsequently Bornträger[64] studied this compound and termed it "rutinic acid," believing it to be acid in nature from its ease of solution in alkaline media. Rochleder and Hlasiwetz[614] isolated rutin from capers (*Capparis spinosa*) and identified it with the "rutinic acid" of Bornträger. At a later date Hlasiwetz[325] came to the conclusion that rutin was identical with the quercetin rhamnoside, quercitrin, but Zwenger and Dronke[803] showed that this could not be the case because on hydrolysis rutin gave quercetin and two molecules of sugar.

At this time there was much confusion concerning the composition of quercetin, to which Liebermann and Hamburger[450] had assigned the formula $C_{24}H_{16}O_{11}$. In an extended series of studies Herzig[321] first adopted this formula but later arrived at the conclusion that quercetin was $C_{15}H_{10}O_7$, a choice supported by work of Perkin and Pate.[570] With this information Schunck[660,661] considered the formula for rutin to be $C_{27}H_{32}O_{16}\cdot 2H_2O$ and stated that on hydrolysis rutin was converted to quercetin ($C_{15}H_{10}O_7$) and two molecules of rhamnose ($C_6H_{14}O_6$).

It was not until 1896 that Schmidt[643] ascertained that the sugar moiety of rutin consisted of one molecule each of glucose and rhamnose and that the correct formula was $C_{27}H_{30}O_{16}$[27]. This early history of rutin has been reviewed in detail by Perkin and Everest[569] and by Charaux.[95]

Rutin and related flavonols were formerly used as dyestuffs for textile fibers but were displaced by the advent of the synthetic dyes. Today only small quantities of the flavonols, quercetin and quercitrin (in the form of orange and lemon flavine) are utilized as pigments.

Occurrence

Rutin has been found to be widely distributed in the plant kingdom and at present is known to occur in at least 34 plant families and 77 plant species.[402] Thirty-three of these are tabulated (Table I) in alphabetical

TABLE I

Plants Containing Rutin

Family	Genus and species	References	Rutin content, %[a]
APOCYNACEAE	*Nerium odorum* Lam.	525	—
ARALIACEAE	*Hedera helix* Linn.	96	—
BETULACEAE	*Betula humilis* Schrank (Swamp birch)	336	1.4, L
BORAGINACEAE	*Lithospermum officinale* Linn.	96	—
CAPPARIDACEAE	*Capparis spinosa* Linn. (Capers)	72, 96, 325, 614, 644, 797	0.32 (72)
CAPRIFOLIACEAE	*Sambucus canadensis* Linn. (Elder)	382, 631, 754	0.77, F (631); 3.5, L (754); 5.2, IF (754); 3.0, MF (754)
	Sambucus nigra Linn. (*S. vulgaris* Lam.) (Elder)	96, 454	—
CRASSULACEAE	*Bryophyllum calycinum* Salisb. (*B. pinnatum*, Kurg)	759	L
	Sedum acre Linn.	512	—
CRUCIFERAE	*Bunias orientalis*	366	—
EMPETRACEAE	*Empetrum nigrum* Linn. (Smokeberry, crowberry)	352	—
EUPHORBIACEAE	*Mallotus japonicus* Muell Arg.	680	—
GLOBULARIACEAE	*Globularia alypum* Linn.	746, 797	2.5, F (746)
	Globularia vulgaris Linn.	746, 797	—
HIPPOCASTANACEAE	*Aesculus californica* Nutt (Pavia C.)	96	—
LEGUMINOSAE	*Daviesia latifolia* R. Br. (Native hopbush)	581	—
	Sophora japonica Linn. (Chinese scholar tree, Japanese pagoda tree)	138, 219, 481, 710, 717, 735	2.4, F (735); 16.3–22.9, F (138)
	Tephrosia purpurea Pers. (Ash vetch)	108a	2.5
LILIACEAE	*Asparagus officinalis* Linn.	88, 173, 524, 719	1.01, MP (719)
MAGNOLIACEAE	*Magnolia grandiflora* Linn.	575	—
	Magnolia kobus DC.	307	—
	Magnolia macrophylla Michx.	455	—
	Magnolia obovata Thumb	575	—
	Magnolia soulangeana Soul	575	—
	Magnolia stellata Maxim	575	—
	Magnolia thompsoniana Hort.	575	—
	Magnolia umbrella Lamb.	455	—
	Magnolia yulan Desf.	575, 772	2.4, F (575)
MYRTACEAE	*Eucalyptus delegatensis*	344	3.0–4.0
	Eucalyptus macrorrhyncha F.v M.	400, 468, 568, 616, 645, 695, 696	10.0, L (696); 13.7–23.1, L (400); 6.0–24.0, L (616)
	Eucalyptus youmani B. and McK.	616	6.8–11.0, L
OLEACEAE	*Forsythia fortunei* Rehd.	522	2.08–4.29, F
	Forsythia pendulata Linn.	262	0.36, F
	Forsythia suspensa Vahl.	210, 522	1.09, F (522)
PALMAE	*Dactylifera palma* Linn. (Date palm)	193	0.36, PG
PAPAVERACEAE	*Eschscholtzia california* Cham.	630	5.0, F
	Hypecoum pendulum Linn.	96	—
PAPILIONATAE	*Onobrychis sativa* Lam.	62	0.3–0.4 P
POLYGONACEAE	*Fagopyrum cymosum*	234, 350	4.0 (May); 8.5 (Oct.)
	Fagopyrum emarginatum	140, 776	2.0–4.5, P (776)
	Fagopyrum esculentum Mnch. (*Polygonum fagopyrum* Linn.) (Japanese buckwheat)	71, 137, 346, 459, 657, 658, 659, 776, 796	0.11, L (658); 1.78, L (796); 0.71, F (796); 2.0+, F (796); 1.16–6.37, L, F (137)
	Fagopyrum tataricum Gaertn. (Tartary buckwheat)	140, 459, 776	3.4–5.0, P (776)
	Fagopyrum tetra-tataricum S.	140, 776	3.0–5.4, P (776)
	Muehlenbeckia chilensis Meissn.	345, 347	2.4, P (347)
	Rheum emodi Wall.	338	0.32, F
	Rheum officinale Baill.	338	1.30, F
	Rheum pruinosum	338	3.10, F
	Rheum rhaponticum L.	338	0.61, F
	Rheum undulatum L.	338	0.70, F
PRIMULACEAE	*Lysimachia vulgaris* Linn. (Common yellow loose strife)	533	—
PROTEACEAE	*Grevillea robusta* Cunn.	387	0.52, L
RHAMNACEAE	*Paliurus aculeatus* Lam. (*Rhamnus paliurus* Linn.)	550	0.15, GF
ROSACEAE	*Prunus melanocarpa* (A. Nels) Rydb. (Wild cherry)	129	1.44–3.88, L
RUBIACEAE	*Galium cruciatum* Linn.	96	—

[a] IF = Immature flowers MF = Mature flowers MP = Mature plant S = Stems F = Flowers L = Leaves P = Plants R = Roots PG = Pollen grains GF = Green fruit PE = Peel

(*continued*)

TABLE I (*continued*)

Plants Containing Rutin

Family	Genus and species	References	Rutin content, %[a]
RUTACEAE	*Citrus hybrid*	397	0.9–3.2, PE
	Ruta chalepensis Vill.	87a	4.56, L
	Ruta graveolens Linn. (Garden rue)	64, 214, 325, 644, 774	2.0, P (214)
SALICACEAE	*Salix triandra* Linn. (*S. amygdalina, β-triandra* L.)	74	0.15–0.70
SANTALACEAE	*Osyris abyssinica* Hochst.	28	—
	Osyris compressa DC. (Cape sumach)	568	—
SAXIFRAGACEAE	*Hydrangea macrophylla*	33	0.36, F
	Hydrangea paniculata (Grandiflora Sieb.)	136	4.1, F
SOLANACEAE	*Lycopersicum pimpinellifolium* (Red Currant Tomato)	220	0.037, L
	Nicotiana glauca	32, 96, 186	1.2–2.1, L (32)
	Nicotiana rustica Linn.	32	0.1–0.7, L
	Nicotiana tabacum Linn.	130, 186, 305, 526, 527, 532	0.008–0.61; avg. 0.40, L (130)
	Solanum angustifolium R. and Pav.	757	0.75, P
	Solanum demissum Lindle	634	—
	Solanum lycopersicum Linn. (L. Esculentum Mill.) (Tomato)	61	—
	Solanum tuberosum Linn. (Potato)	96, 737, 738	0.2, F (738)
TERNSTROEMIACAE	*Thea assam* (Tea)	522a	0.12, L
UMBELLIFERAE	*Bupleurum falcatum* Linn.	592	0.3–0.4, P
	Heracleum spondylium Linn.	96	—
VIOLACEAE	*Viola lutea splendens*	519	16.6, F
	Viola tricolor (Linn.) (Arvensis and vulgaris)	160, 471, 568, 795	0.13, L; 0.08, S; 0.05, R (568); 2.0 (795)
	Viola tricolor Linn., Var. Maxima (Giant Roggli)	519	18.3–21.2, F
	Viola tricolor Linn. (Odorata)	66	—

[a] IF = Immature flowers MF = Mature flowers MP = Mature plant S = Stems F = Flowers L = Leaves P = Plants R = Roots PG = Pollen grains GF = Green fruit PE = Peel

order according to the family name. References are given to the original investigators and to others who have contributed to the identification of the rutin. When available, the percentage rutin content is also given together with the reference to the report containing the information.

In addition to the members of the spermatophytes listed in Table I, rutin has also been isolated from a thallophyte. Kuhn and Löw[410] isolated it from the gametes of a Chlamydomonas mutant, which they termed *Chlamydomonas agametos*.

In the quest for new sources for rutin there will undoubtedly be much duplication of effort, since negative findings are not usually reported. In a search for steroidal saponins, Wall *et al.*[785] are extending the screening of plant extracts to include other constituents among which are flavonoids. In their initial report,[785] the first in a series, of the approximately 1000 plant samples tested from 29 families, (about two-thirds of which were of the genera Agave, Yucca, and Dioscorea) most were devoid of flavonoids, but 33 samples are listed as containing flavonoids in trace amounts and 4 in moderate quantities. The test used in this work was the cyanidin reaction of Willstätter[82,784] which, although not specific for rutin, indicates the presence of flavonoids.

Plants Containing No Appreciable Rutin

During a routine examination at the Eastern Utilization Research Branch many domestic plants, the extracts of which gave a positive cyanidin test, failed to yield rutin by the gravimetric technique of Naghski *et al.*[517] based on the isolation of the flavonoid. This method, however, is not sensitive to small quantities of rutin (less than 0.1%) and so plants containing only traces would escape detection. Those plants in which no rutin was found gravimetrically are listed in Table II.

TABLE II

Plants Containing No Appreciable Rutin

Family	Genus and species	Part examined
ANACARDIACEAE	*Rhus glabra* Linn. (Sumac)	Fresh flowering heads
BALSAMINACEAE	*Impatiens pallida* Nutt. (Jewelweed)	Fresh whole plant
BERBERIDACEAE	*Podophyllum peltatum* Linn. (May apple)	Fresh whole plant
BORAGINACEAE	*Borago officinalis* Linn. (Borage)	Fresh leaves
CANNABINOIDEAE	*Humulus lupulus* Linn. (Hop)	Dried flowers
CAPRIFOLIACEAE	*Viburnum opulus* Linn. (Snowball)	Fresh flowers
CARYOPHYLLACEAE	*Stellaria media* Vill. (Chickweed)	Fresh whole plant

(*continued*)

TABLE II (*continued*)

Plants Containing No Appreciable Rutin

Family	Genus and species	Part examined
CHENOPODIACEAE	*Beta vulgaris* Linn. (Beet)	Fresh tops
	Var. cicla (Swiss chard)	Fresh leaves
	Spinacea oleracea Linn. (Spinach)	Fresh leaf
	Spiraea vanhouttei Zabel (Spirea, Bridal wreath)	Fresh flowers
COMPOSITAE	*Aster novae-angliae* Linn. (New England Aster)	Fresh flowers
	Cichorium intybus Linn. (Chicory)	Fresh white flowers
	Chrysanthemum carinatum Schousb.	Fresh yellow flowers
	Chrysanthemum parenthenium Pers. (Feverfew)	Fresh flowers
	Galinsoga ciliata (Raf.) Blake	Whole plant
	Lactuca sativa Linn. (Lettuce)	Fresh leaf
CONVOLVULACEAE	*Ipomoea batatas* Poir. (Sweet potato)	Fresh vines
CRUCIFERAE	*Brassica arvensis* Kuntze (Mustard weed)	Fresh yellow flowers
	Brassica oleracea Linn. (Var. *acephala*, Kale; var. *Botrytis* Linn., Broccoli; var. *capitata alba* Linn., Cabbage)	Fresh flowering heads and attached leaflets
CUCURBITACEAE	*Curcurbita pepo* Linn. (Pumpkin)	Fresh ripe rind; Fresh blossoms
EQUISETACEAE	*Equisetum hiemale* Linn. (Horsetail or Scouring-rush)	Fresh whole plant
EUPHORBIACEAE	*Euphorbia epithymoides* Jacq. (*E. polychroma* Kern.) (Spurge)	Fresh whole plant
GNETACEAE	*Ephedra viridis* Wats. (Mexican tea)	Dried whole plant
GRAMINEAE	*Holcus sorghum* Linn. (Var. White Hegari)	Fresh whole plant
	Lolium perenne Linn. (Ryegrass)	Fresh whole plant
	Setaria glauca Beauv. (Yellow foxtail)	Fresh whole plant
IRIDACEAE	*Iris pseudacorus* Linn. (Var. Seminole-Yellow flag)	Fresh flowers
LABIATAE	*Coleus sp.*	Fresh plant
	Mentha spicata Huds (Spearmint)	Fresh leaf
LEGUMINOSAE	*Medicago sativa* Linn. (Alfalfa)	Fresh whole plant
	Glycine soja Sieb. and Zucc. (*Phaseolus max* Linn.) (Soybean)	Fresh leaf, less most of stem
	Trifolium repens Linn. (White clover)	Fresh blossoms
LILIACEAE	*Convallaria majalis* Linn. (Lily-of-the Valley)	Fresh leaves and blossoms
MALVACEAE	*Althaea rosea* Cav. (Hollyhock)	Fresh flowers
	Gossypium hirsutum Linn. (Cotton)	Dried leaves
	Malva rotundifolia Linn. (Common mallow)	Fresh whole plant
MORACEAE	*Broussonetia papyrifera* Vent. (Paper-mulberry)	Fresh flowers
MYRTACEAE	*Eucalyptus bauer-iana* Schau. / *E. botryoides* Sm.	Dried leaves
	E. cornuta Labill / *E. corynocalyx* F.v.M.	Dried leaves
	E. costata Br. aff. / *E. crebra* F.v.M.	Dried leaves
	E. eugenioides Sieb. / *E. ficifolia* F.v.M.	Dried leaves
	E. globulus Labill / *E. goniocalyx* F.v.M.	Dried leaves
	E. leucoxylon F.v.M. / *E. paniculata* Sm.	Dried leaves
	E. polyanthemos Schau. / *E. robusta* Sm.	Dried leaves
	E. rostrata Schl. / *E. rudis* Endl.	Dried leaves
	E. salmonophloia F.v.M. / *E. sideroxylon* Cunn.	Dried leaves
	E. tereticornis Sm. / *E. viminalis* Labill.	Dried leaves
PHYTOLACCACEAE	*Phytolacca decandra* Linn. (Pokeweed)	Fresh leaves
POLYGONACEAE	*Eriogonum giganteum*	Dried whole flowers
	Fagopyrum esculentum Linn.	Buckwheat seed and honey
	Polygonum perxicaria Linn. (Smartweed, ladysthumb)	Fresh whole plants
	Rheum rhapanticum Linn. (Rhubarb, Pieplant)	Fresh leaf
	Rumex crispus Linn. (Yellow dock)	Fresh plant
	Rumex hymenosephalus Torr. (Canaigre)	Dried root
PORTULACACEAE	*Portulaca oleracea* Linn. (Pusley)	Fresh whole plant
RANUNCULACEAE	*Paeonia sp.* (White peony)	Fresh flowers
	Ranunculus bulbosus Linn. (Common field buttercup)	Fresh flowers
ROSACEAE	*Prunus serotina* Ehrh. (Wild black cherry)	Dried leaves
RUTACEAE	*Citrus aurantifolia* Swingle (*Limonia aurantifolia* Ch.) (Lime)	Fresh immature peel and fruit
	Citrus grandis Osbeck (*Citrus decumana* Linn.) (Grapefruit)	Fresh mature peel and fruit
	Citrus limonia Osbeck (Lemon)	Fresh immature peel and fruit
	Citrus sinensis Osbeck (Common orange)	Fresh mature peel and fruit
SAXIFRAGACEAE	*Hydrangea arborescens* Linn.	Flowering heads and stipules only in early bud
	Philadelphus coronarius Linn. (Mock orange)	Fresh blossoms
SOLANCEAE	*Capsicum annum* Linn. (Pepper)	Fresh immature fruit
	Lycopersicon esculentum Mill. (*Solanum lycopersicum* Linn.) Var. Stone (Tomato)	Vine and fruit (green and ripe)
UMBELLIFERAE	*Daucus carota* Linn., Var. sativa (Wild Queen Anne's-lace)	Flower head
	Petroselinum hortense Hoffm. (Parsley)	Fresh leaf
URTICACEAE	*Ramium niveum* Linn. (Ramie)	Fresh tops and leaves
VIOLACEAE	*Viola papilionacea* Pursh (Common violet)	Fresh white flowers

Characterization of Rutin

Microcrystallography. Rutin is a pale yellow, tasteless powder consisting of microscopic needle-shaped crystals. When rutin crystallizes from aqueous extracts, the needles have a tendency to arrange themselves in fan-shaped aggregates as illustrated in Figure 2. This is especially characteristic when the solutions have not been clarified sufficiently to remove all particles which can act as foci for crystallization. Pure rutin crystallizes from solution in distilled water as isolated needles. It crystallizes from anhydrous methanol and ethanol with three molecules of solvent in the form of large fusiform plates. Under proper conditions these can be made to grow to exceptional size. Figure 3 shows a photograph of the crystals obtained by the spontaneous concentration of an absolute ethanol solution in a petri dish.

Keenan[377] found that rutin crystals consisted of minute rods and were too small for satisfactory microscopic examination. However, by crystallizing from alcohol, he was able to obtain larger rods which contained 1 molecule

TABLE III

Refractive Indices of Rutin

Flavonol	α	β	γ	ω	ϵ
Rutin ($C_{27}H_{30}O_{16}\cdot CH_3CH_2OH$)	1.508	1.734	1.734	—	—
Quercetin ($C_{15}H_{10}O_7\cdot 2H_2O$)	1.555	1.734	1.734	—	—
Quercitrin ($C_{21}H_{20}O_{11}\cdot 2H_2O$)	—	—	—	1.508	1.734

of solvent of crystallization. (The strength of alcohol or the drying conditions were not given so that these data cannot be compared with those of Krewson and Naghski.[401]) The microcrystallographic properties of these crystals are described as follows:

"With crossed nicols, the extinction is parallel and the sign of elongation is negative. The rods were not large enough to reveal interference figures with the microscopic examination. The significant refractive indices are: $\alpha = 1.508$ (commonly shown lengthwise on rods), $\beta = 1.734$ (commonly shown crosswise and apparently the β-value although an interference figure could not be obtained), $\gamma = 1.734$. All $\pm$ 0.002." Similar information was also obtained for quercitrin and the aglycone quercetin. A comparison of the refractive indices for the three compounds are presented in Table III.

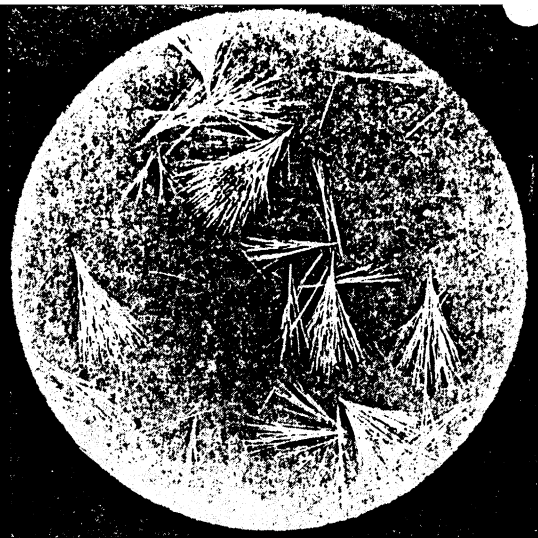

Fig. 2. Rutin crystals, magnified 213×.

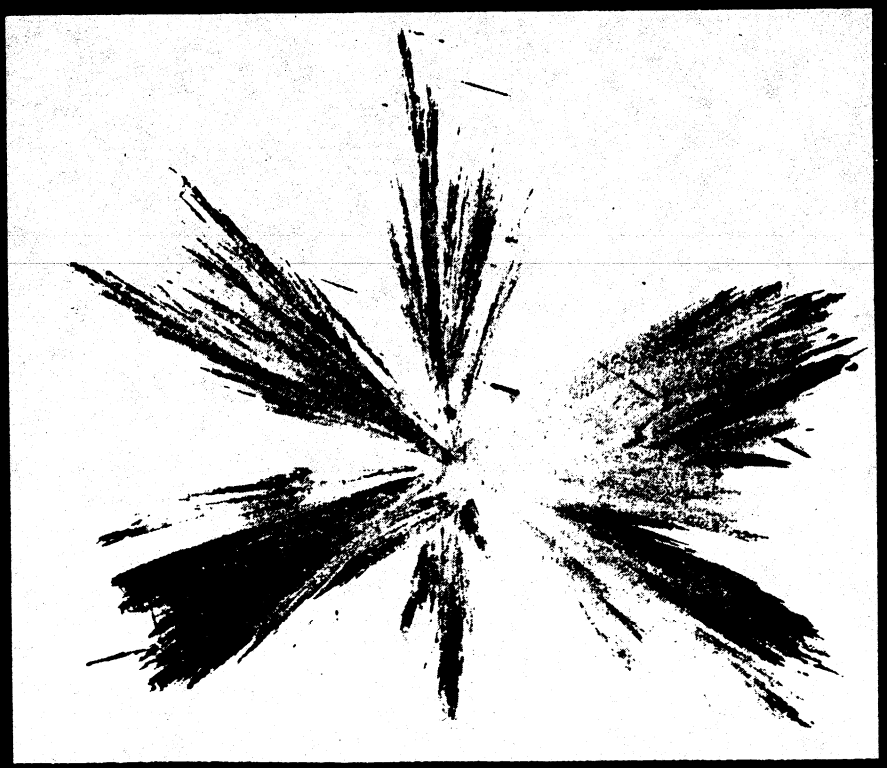

Fig. 3. Crystals of rutin ethanolate, magnified 3×.

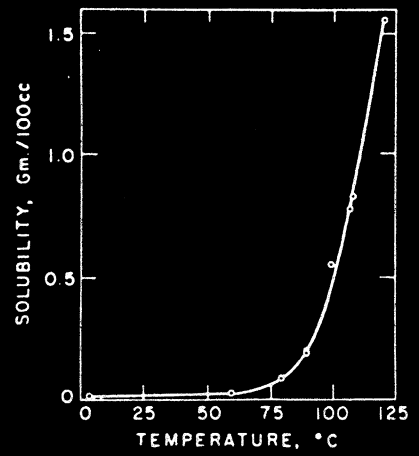

Fig. 4. Approximate solubility of rutin in water.

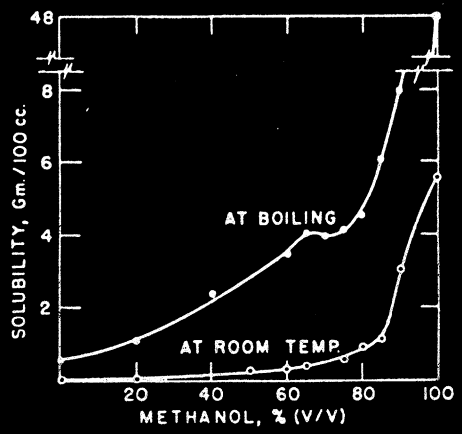

Fig. 5. Approximate solubility of rutin in aqueous methanol.

Solubility. The solubility of rutin in water and in various solvent-water solutions has been determined by Krewson and Naghski.[401] It is soluble in boiling water in the proportion of 5 to 6 grams per liter, but much less soluble in cold water (about 0.1 gram per liter). It readily crystallizes from boiling water solutions on cooling. The solubility in water is influenced greatly by the temperature especially near the boiling point (Figure 4). Elevating the temperature to 121°C. by increasing the pressure to 15 pounds per square inch increases the solubility three-fold over the solubility in boiling water at standard atmospheric conditions.

The solubilities of rutin in methanol, ethanol, isopropanol, acetone, and in their water solutions at room temperature and near the boiling point are shown in Figures 5–8. The solubility curves for these solvents show two maxima, one occurring in the anhydrous solvent, and the other at an organic solvent concentration ranging from 70–98 percent.

Rutin is exceedingly soluble in such hot solvents as methanol, ethanol, *n*-butanol, glycerol, the glycols, 1-4 dioxane, pyridine, morpholine, and water solutions of the inorganic bases; it is moderately soluble in such hot solvents as acetone, the propanols and glacial acetic acid; it is insoluble in hydrocarbons, chlorinated hydrocarbons, nitroparaffins, and ethers. Table IV shows the solubility of rutin in some of the more important solvents.

TABLE IV

Approximate Solubilities of Rutin in Water and Various Anhydrous Solvents at Room Temperature and Near the Boiling Point

Solvent	Solubility at Room temperature Gm./100 cc.	Solubility at Near boiling temperature, Gm./100 cc.
Water	0.013	0.50–0.55
Methanol	5.5	48.0
Ethanol	0.55	36.0
Isopropanol	1.45	7.0
n-Propanol	0.60	4.0
n-Butanol	—	70.0
Acetone	0.56	2.0
1-4 Dioxane	—	13.6
Pyridine	37.3	—
Acetic acid	—	9.2

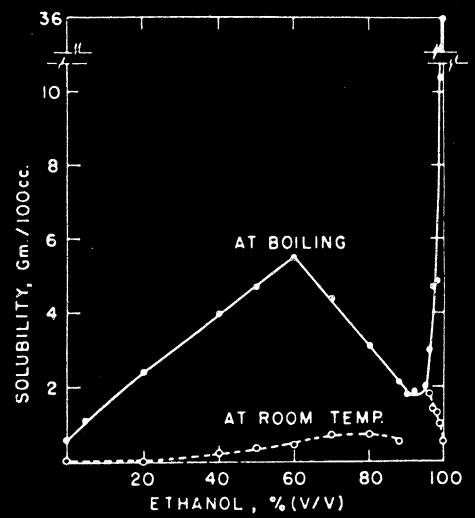

Fig. 6. Approximate solubility of rutin in aqueous ethanol.

Numerous iron salts have been tested[399] to determine their ability to increase the solubility of rutin in water. Ferrous ammonium sulfate, ferric chloride, ferrous gluconate, ferric ammonium sulfate, and ferrous lactate solubilize rutin by reacting in a manner which appears to be stoichiometric. Also, rutin was found to be solubilized by colloidal saccharated ferric oxide.[396,399] Ferric sulfate, ferric ammonium citrate, ferric glycerophosphate, ferrous phosphate, and ferrous chloride failed to solubilize rutin.

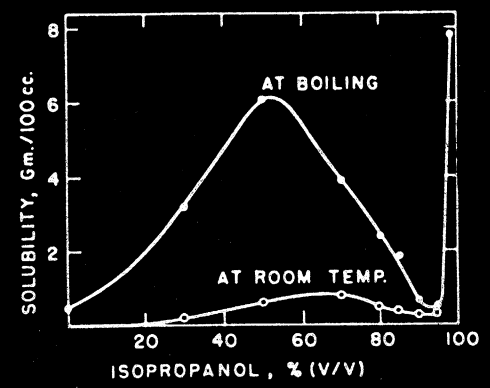

Fig. 7. Approximate solubility of rutin in aqueous isopropanol.

Cuprous chloride solubilizes rutin; but cupric sulfate, cobaltous chloride, manganous chloride, and manganous sulfate do not.

The solubilizing properties of alkali, methyl glucamine, piperazine, propylene glycol, and hexamethylenetetramine have been used to prepare solutions of rutin for intravenous injections.[14a,106,305,576,613,751]

Other methods for increasing the solubility of rutin have involved the synthesis of an alkyl carbonate derivative;[544,734] complexing with sodium borate[392,681,683] and the addition of aliphatic amines, alkali salts of amino acids or amino sulfonic acids.[171,235,523,542,583,682]

Solvate Formation. Rutin crystallizes from water with three molecules of water of hydration which are removed with varying degrees of ease. Perkin[567,568] found that rutin dried over concentrated sulfuric acid for three weeks lost 5.76 percent water ($2H_2O$ = 5.44%); after additional drying at

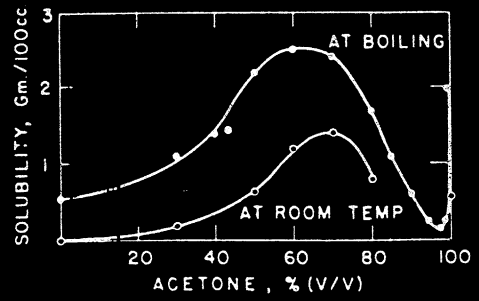

Fig. 8. Approximate solubility of rutin in aqueous acetone.

130°C. it lost 1.49 percent ($1/2H_2O$ = 1.43%) and finally after drying at 160°C. it lost an additional 1.67 percent ($1/2H_2O$ = 1.47%) making a total of 3 molecules of water. Recently, Naghski *et al.*[521] determined the degree of hydration of rutin by means of vapor phase absorption studies. Figure 9 shows the moles of water absorbed at various degrees of relative humidity by two rutins of different qualities, and by quercetin. Highly refined rutin absorbed up to 3.1 moles of water when exposed to higher humidities while

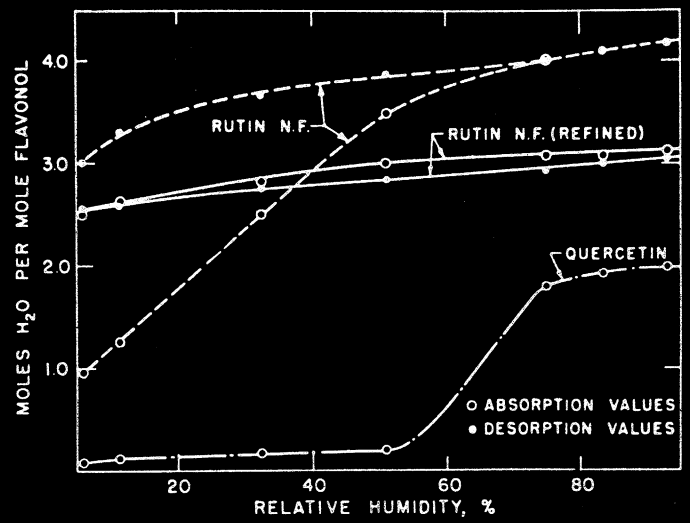

Fig. 9. Water sorption isotherms for rutin and quercetin showing degree of hydration in relation to relative humidity.

N.F. rutin absorbed up to 4 moles of water. It is suggested that the presence of small amounts of impurities disrupts the crystal structure and permits the accumulation of free water. The absorption curves indicate that the highly refined rutin is somewhat more hygroscopic than the N.F.; however, both rutins show little loss of moisture during desorption until extremely low humidities are reached. These results demonstrate clearly why it is difficult to obtain an anhydrous sample of rutin, and have been used for the improvement of the methods for determination of moisture in this highly hygroscopic material.[521]

As previously mentioned, pure rutin crystallizes from solution in near anhydrous methanol, ethanol, and acetone also with three moles of solvent.[521] The crystals are many times larger than those obtained from water (Figure 3). These crystals dissolve readily in cold water and after a

short period the solutions precipitate the characteristic hydrate. The rutin crystals solvated with alcohol are bitter to taste (undoubtedly a result of the increased solubility) which is in accord with expectations for a substance with a glycosidic nature.

Optical Rotation. The optical rotation of rutin in various solvents has been recently studied extensively by Sosa and Plouvier,[708] and Krewson and Naghski.[401] Rutin is optically active and is levo-or dextrorotatory depending upon the solvent used and the concentration of water in the solvent. Rutin is dextrorotatory in neutral and acidic solvents. Dilution of the solvents with water decreases the rotation; in 50% concentration

TABLE V

Optical Rotation of Rutin (Moisture Content 7.7%) in Various Solvents and Solvent-Water Mixtures

Solvent	Solvent Strength % (by wt.)	Optical Rotation $[\alpha]_D^{24°}$ ± 0.5° C. at Rutin Concentration of		
		0.5000 gm./100 cc.	1.000 gm./100 cc.	2.000 gm./100 cc.
Ethanol	99.7	+11.46	+12.19	+13.26
Ethanol	95.0	+ 6.77	+ 7.58	—[a]
Ethanol	49.9	− 9.10	—[a]	—[a]
Methanol	99.8	+ 4.63	+ 5.35	+ 6.68
Methanol	49.9	− 1.52	—[a]	—[a]
Isopropanol	98.2	+ 3.52	—[a]	—[a]
Isopropanol	49.1	− 4.60	—[a]	—[a]
n-Butanol	99.9	+20.77	+18.46	+18.25
Acetone[b]	49.6	−16.03	−11.02	—[a]
1-4 Dioxane	100.0	+21.37	—[a]	—[a]
1-4 Dioxane	50.0	− 9.21	−14.73	
1-4 Dioxane[c]	50.0		−11.98	−12.52
Pyridine[d]	100.0	−38.35	−38.83	−40.01
Pyridine	80.0	−41.98	−47.10	−48.12
Pyridine	25.0	−33.47	−34.56	−34.06
Acetic Acid	99.8	+42.98	+42.25	+37.97
Acetic Acid	49.9	− 3.63	—[a]	—[a]

[a] Blanks (—) indicate rutin not soluble to this extent.
[b] Acetone, 99.1%, would not dissolve this rutin in sufficient quantity to make a 0.5% solution.
[c] Values with technical grade 1-4 dioxane.
[d] Since pyridine-rutin solutions react with metals, contact with metal parts of the polarimeter tube must be avoided.

rotation is levorotatory. Rutin is levorotatory in the alkaline solvents. Table V presents representative data on the optical rotation of rutin in various solvents. Of the solvents used, pyridine (80–100%) and glacial acetic acid give the greatest angle of rotation and so appear to be the best for measuring the rotation; n-butanol is a third choice.

Hydrolysis. On hydrolysis with dilute acids rutin yields a molecule each of the flavonol, quercetin; and the sugars, glucose and rhamnose[613] which is represented by equation as follows:

$$\underset{\text{Rutin}}{C_{27}H_{30}O_{16}} + 2H_2O \rightarrow \underset{\text{Quercetin}}{C_{15}H_{10}O_7} + \underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Rhamnose}}{C_6H_{12}O_5}$$

In the rutin molecule the sugars are present as a disaccharide, rutinose, which may be obtained either by mild hydrolysis with acetic acid[809] or by an enzyme derived from *Rhamnus utilis*.[97] Rutin is not hydrolyzed by α-glucosidase or by emulsin.[532a] When rutinosides are decomposed with ozone in acetic acid, a crystalline rutinose acetate is obtained which can be

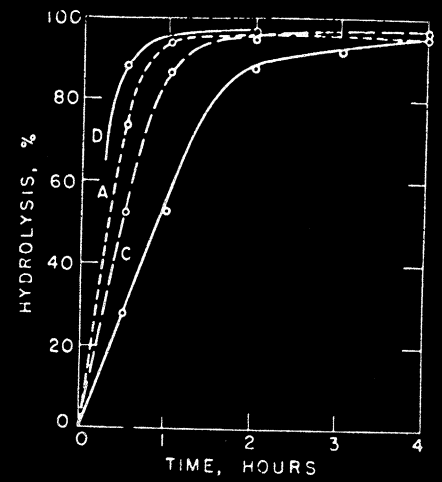

Fig. 10. Effect of concentrations of rutin, acid, and size of batch on rate of hydrolysis of rutin by boiling aqueous sulfuric acid. *A:* 10% rutin, 5% acid, 2000 ml. volume, *B:* 10% rutin, 2% acid, 2000 ml. volume, *C:* 5% rutin, 2% acid, 2000 ml. volume; *D:* 5% rutin, 2% acid, 150 ml. volume.

used to identify the sugar moiety.[494] Recently Fox *et al.*[223] have found that the rhamnose moiety can be preferentially removed from rutin by refluxing with formic acid in cyclohexanol.

Conditions for the hydrolysis of rutin to quercetin with sulfuric acid as a catalyst have been investigated by Naghski and Krewson.[518] The rates of hydrolysis of 2 to 10 percent slurries of rutin with boiling aqueous sulfuric acid of varying strength are presented in Figure 10. The data obtained indi-

cate that rutin is hydrolyzed rapidly during the early part of the reaction, but the curve becomes asymptotic as the reaction approaches completion. Increasing the acid concentration or reducing the rutin concentration increases the rate of hydrolysis. These results indicate that rutin is more than 95 percent hydrolyzed by use of 2 percent rutin slurries heated one hour with both 2 and 5 percent solutions of sulfuric acid. More concentrated slurries of rutin require up to four hours heating to approach the same degree of hydrolysis.

Melting Point and Color Tests. The melting point is not a good criterion of purity in the case of rutin since this compound melts over a substantial range of temperature (5–7°) even when highly purified and containing no contaminants as shown by paper chromatography. This was found to be true of rutin prepared from various plant sources such as tobacco, buckwheat, elder, Sophora, Eucalyptus, yellow pansies and many others.

Examination of the hydrolytic products is a more reliable means of identification. The quercetin has a melting point of 312–314°C. and its pentaacetyl derivative melts at 194–196°C. The two sugars can be identified as the phenylosazones and by paper chromatography.[580]

A valuable method of characterization and analysis is the determination of the absorption spectrum in the ultraviolet. Rutin shows maxima at 247.7 and 362.5 mμ with absorptivities (specific extinction coefficients) of 38.0 and 32.6 liter gm^{-1} cm^{-1} respectively. However, in the use of the absorption spectrum, it is necessary to keep in mind that quercetin, quercitrin and other quercetin derivatives have spectra similar to that of rutin (see also the section on rutin preparation).

Various color tests[593] have been used for the detection of rutin but most flavones, flavanones and flavonols when tested in this way give similar findings. For example, rutin dissolves in alkaline solutions with the formation of an intense yellow color.[168] With alcoholic or aqueous ferric chloride, rutin gives an intense green color. With acid and magnesium in alcoholic solution, rutin gives a red color. Bryant[82] states that this color reaction for flavonoids was first used by Willstätter[784] and that "It was later established that this color was due to the presence of a γ-benzo-pyrone nucleus." Valentin and Wagner[762] have made this reduction the basis of a colorimetric assay procedure for the determination of rutin in tablets and an approximation of rutin in plant decoctions and extracts.

Rutin forms colored complexes with the salts of many heavy metals.[777] This property is made use of for its analytical determination.[170,294,332,579] Complexing with metals produces a shift in the absorption maximum from the near ultraviolet to the visible. Figure 11 shows the spectral absorption curve of rutin and of the rutin-aluminum chloride complex. The aglycone

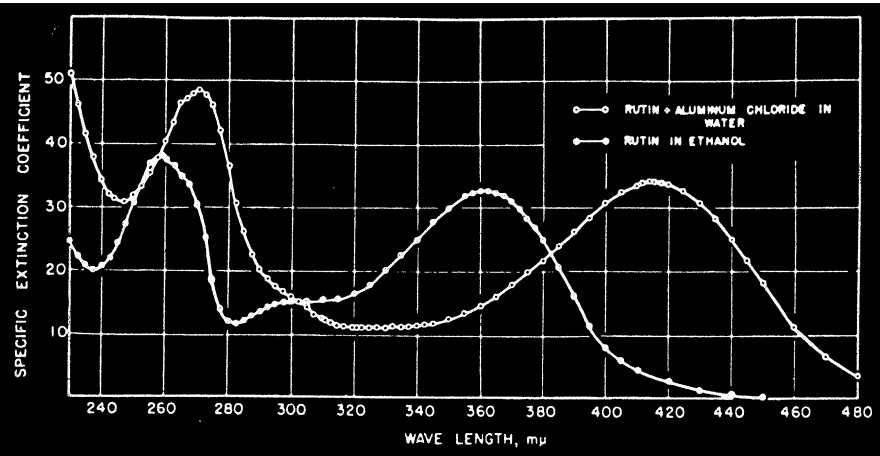

Fig. 11. Absorption spectra for rutin in 95% ethanol and for the rutin-aluminum chloride complex in water. The absorptivities (specific extinction coefficients) for both curves are based on 1 g. of rutin/l. in a 1-cm. cell.

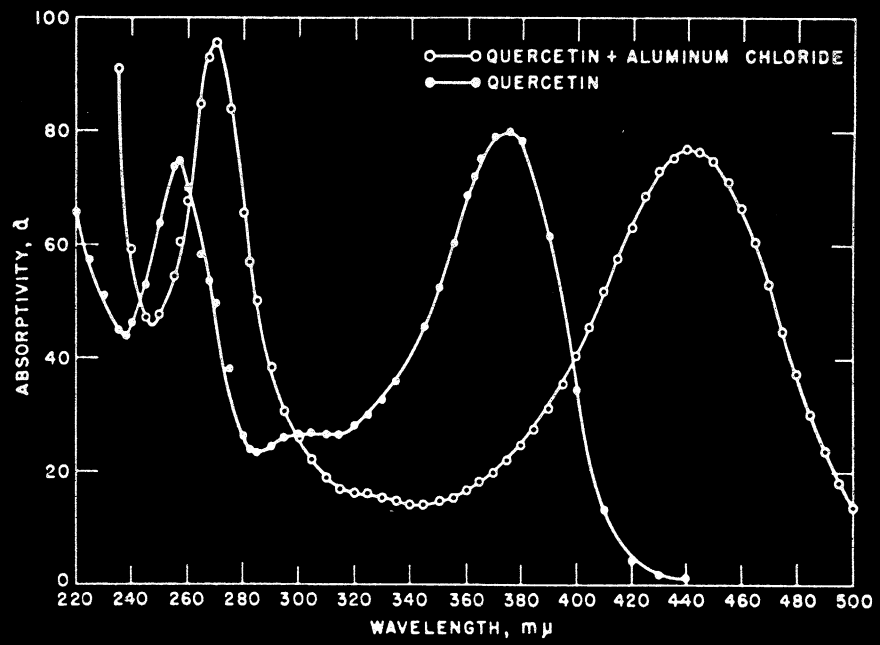

Fig. 12. Spectral absorption curves for quercetin in 95% ethanol and for quercetin + aluminum chloride in potassium acetate buffered aqueous ethanol.

of rutin (quercetin) exhibits a similar shift in the position of the absorption maximum as a result of complexing with aluminum chloride (Figure 12). Hörhammer and Hänsel investigated the complexing of rutin and quercetin with zirconium perchlorate, aluminum chloride and cupric sulfate, and found a stoichiometric relationship.[333,334] Tomicek and Holecek[749] used morin and quercetin for testing and gravimetric estimation of niobium and tantalum. The ability to chelate with metals such as iron and copper, a property common to the flavonoids, may also be responsible in part for the physiological activity.[101,103]

Rutin forms a fluorescent borocitric acid complex. Techniques based on this have been advocated for its quantitative determination[259,785] but are not entirely satisfactory. Hörhammer, Hänsel and Strasser[335,337] prepared the quercetin boric-oxalic acid complex and obtained it in crystalline form.

Chromatography. The use of paper chromatography is a valuable technique in the detection of rutin. Studies on the paper chromatographic behavior of the flavonoids have been published by Wender *et al.*[246,775] and by Bate-Smith and Westall.[39] Gage, Douglass and Wender[244] have been able to differentiate among a wide variety of compounds by this technique. The R_f value (ratio of distance traveled by the pigment to distance traveled by the solvent) was found to be characteristic, using different solvents and amounts of pigment as little as 10 μg. Colors produced in white light with and without chromogenic sprays, and fluorescence produced under the ultraviolet lamp, serve as a further means of identification.[90,231] A technique using round filter papers has also been reported.[536,537]

Bradfield and Flood[70] describe a method by which the ultraviolet absorption spectra of substances can be determined while on the paper chromatogram. This aids in the identification of flavonoids which may have similar R_f values but different absorption spectra.

Gage and Wender[247] determined quantitatively the flavonol-3-glycoside by elution from paper chromatograms with aqueous aluminum chloride solution and spectrophotometric estimation of the colored complex formed. Quercetin could not be recovered quantitatively because of the adsorption of some of this complex by the paper.

In a recent method[516] quercetin was separated from rutin by using a short filter paper strip and allowing the rapidly migrating quercetin to travel off the chromatogram into a receiver.

The property of ion exchange resin to absorb flavonoids from aqueous solutions has been utilized by Wender and coworkers to isolate trace amounts of these compounds from plants.[245,504,778,779,780,781,782] They have also found that purification and separation of flavonoids can be accomplished using columns of magnesol as the absorbent.[348]

Paper electromigration has also been found applicable.[372]

Analytical Procedures for the Determination of Rutin in Plants

Recently two methods have been proposed for the determination of rutin in plants.[517,754] One of these[517] is a gravimetric procedure which depends on the extraction, and isolation of the glycoside. In general it is adequate but suffers from the inevitable inaccuracies inherent in isolating and weighing a plant constituent. However, if performed carefully, it is sufficiently precise to afford reliable information on the rutin content of material under investigation. It offers the advantage that the isolated product can be characterized, an important consideration when dealing with a new plant source. Difficulty is usually encountered with this method in the analysis of plant materials containing small or trace amounts of rutin, because the glycoside either fails to crystallize, or does so incompletely when it is present in small quantities. This is especially true if the extracts contain much sugar and become sirupy upon concentration.

The other method[754] is an absorptiometric method which determines the concentration of rutin by measurement of the color produced when rutin complexes with aluminum chloride. This method offers a great reduction of time required to complete the analysis as well as greater precision and accuracy than the gravimetric method. It is not specific for rutin since other flavonols yield similar colored complexes with aluminum chloride.

Since both methods have value, each will be described.

Gravimetric Method

Rutin in Fresh Plant. Select approximately 200 grams of whole plant (weighed to the nearest 0.1 gram) for each rutin determination, and cut into pieces 1 to 2 inches long. Transfer to an extra large Soxhlet extractor as quickly as possible after cutting, using a thin layer of cotton or glass wool to prevent plant material from entering the siphon tube. Immediately add sufficient ethanol (absolute) for extraction (a liter flask requires about 600 ml.) and extract for 2 to 4 hours. At the end of this period, replace the flask containing the ethanol extract with one containing fresh ethanol (to prevent loss of rutin from prolonged heating, since the major portion is extracted in this period) and continue extraction until the extract is colorless (approximately 8 to 12 hours). Combine the extracts in a large casserole and evaporate on a steam bath until all the alcohol is removed (do not evaporate to dryness; add water if necessary). Add enough water to dissolve the rutin (100 ml. for each 0.4 gram expected), boil vigorously for 1 to 2 minutes and filter through a rapid filter paper. Transfer the filter paper to the casserole, boil with a small quantity of water, and refilter. Repeat if necessary until all rutin is dissolved. Store the combined filtrates at room temperature overnight and then in a refrigerator until crystal-

lization is complete (1 to 2 days). Filter through a tared Gooch crucible, wash with cold water, and dry at 110°C. for 4 hours, or to constant weight. Cool, weigh, and calculate as percent crude rutin.

Rutin in Dried Plant. Place a sample of ground buckwheat containing between 0.5 and 0.75 gram of rutin (15 to 20 grams or 100 grams of low-rutin materials) into a Smalley type extractor, place a plug of cotton on top and bottom of sample layer. Extract with ethyl ether for 8 to 12 hours (to remove fats and carotenoids). Remove the ether by drawing air through the sample and extract for 4 hours with absolute ethanol. Replace ethanol with fresh ethanol and extract for an additional 4 to 6 hours. Again change the ethanol and continue extraction for a total of 16 hours. Combine the extracts in a casserole and evaporate until the ethanol is removed (do not evaporate to dryness; add water if necessary). Add sufficient water to dissolve the rutin (100 ml. for each 0.4 gram expected), boil vigorously for 1 to 2 minutes (add 0.25 to 0.50 gram of barium chloride if coagulation of non-rutin materials does not occur with boiling) and filter through a rapid filter paper. Transfer the filter paper to the casserole and boil with 25 to 50 ml. of water. Filter. Repeat if necessary until all the rutin is dissolved. Determine rutin in filtrates as described under "fresh plant."

Correction of Rutin Value by Refining. The crude rutin obtained by this procedure contains 0.5 to 15 percent impurities. Consequently the rutin values are usually high, and the wide variation in content of impurities makes it impossible to use a correction factor. A study of the impurities showed that the major portion could be divided into two types—"benzene soluble" and "alcohol insoluble." To correct the crude rutin values, the following purification technique was developed. It is somewhat tedious and time consuming, but is justified where greater accuracy is required. The much simpler method of purification by recrystallization from solvents is unsatisfactory since it involves uncontrollable loss of the glycoside.

Procedure: The Gooch crucible containing the crude rutin is placed in a Soxhlet extractor and extracted with dry benzene for 12 hours. The benzene extract is concentrated to about 10 ml. and transferred to a tared beaker. The extract is then evaporated to dryness on a steam bath, and finally dried at 110°C. for 0.5 to 1 hour. The residue is weighed and calculated as percent "benzene solubles."

The Gooch crucible containing the benzene-extracted crude rutin is dried to remove the benzene, and extracted with boiling absolute ethanol. This is best accomplished by placing the crucible over a suction flask, adding a small quantity of boiling ethanol and triturating the cake of rutin with the flat end of a short stirring rod, being careful not to disrupt the asbestos mat. The solvent is drawn through slowly with vacuum, and the process is repeated several times until all the rutin is dissolved, the volume of solvent being kept as low as possible (40 to 50 ml.). The crucible is then transferred to another suction flask, and the extract is heated to boiling and filtered through the asbestos mat, in order to recover any asbestos fibers that may have been dislodged, and also any insoluble materials that may have worked through the mat. The crucible is then rinsed with a few ml. of fresh solvent to remove any entrained extract, and dried at 110°C. for 2–4 hours. The difference between the new weight and the original tare weight is calculated as "alcohol insolubles." Subtract the sum of "benzene solubles" and "alcohol insolubles" from the weight of crude rutin to get the "corrected" weight of rutin.

Spectrophotometric Method

Extraction of Ground Meal. A 2-gram sample of the ground meal is distributed evenly over the surface of a piece of absorbent cotton, approximately 4 × 2 × 0.25 inches. The cotton is rolled along its major axis and placed in a Smalley extractor, the bottom of which contains a small plug of cotton. The extractor is fitted to the extraction flask, which contains a few glass beads, and approximately 75 ml. of absolute ethanol is poured through the cotton in the extractor. (Ethanol is preferred to methanol because ethanol dissolves less non-rutin colored plant material.) The sample is extracted for 6 hours. The cooled extract is diluted to 250 ml. with isoamyl alcohol.

Extraction of fresh plant is carried out as described in the gravimetric procedure. The extract is made to a convenient volume either by concentration or dilution and an aliquot (50 to 75 ml.) is diluted to 250 ml. with isoamyl alcohol.

Isolation of Rutin. A 20-ml. aliquot of the isoamyl alcohol solution is placed in a 125-ml. Squibb separatory funnel and extracted with three 25-ml. portions of 0.1 *M* aluminum chloride. During this operation, the rutin passes into the aqueous phase as the yellow rutin-aluminum chloride complex, leaving the other extracted plant pigments in the isoamyl alcohol layer. After each shake-out, the solvents are separated by centrifugation, and the lower aqueous layer is run off into a 250-ml. volumetric flask. The combined aqueous extracts are diluted to 250 ml. with distilled water.

Absorptiometry. The rutin-aluminum chloride complex has an absorption maximum at 416 mμ. The absorbance of the solution at its final dilution is determined with a Beckman DU spectrophotometer at 416 mμ, versus a correspondingly diluted aluminum chloride blank, using 1-cm. cells. No appreciable absorption has been observed in blank analyses of the alcohol reagents or of extracts from the cotton, alone or in combination with the aluminum chloride solution. Appropriate dilutions are made

to maintain the absorbance between 0.2 and 0.8. Through this range the solutions follow Beer's law accurately. The weights and dilutions given are suitable for materials having rutin concentrations of 1 to 4%. The rutin complex is stable, and the absorbance may be determined almost immediately and remains unchanged for at least 2 hours. Glass or interference filter absorptiometers isolating the 416 mμ region can be used, but for each individual instrument the absorptivity factor or a calibration curve would have to be established.

Replicate analyses of pure rutin (rutin·$3H_2O$)[742] have established its absorptivity, a, as 30.7 under the conditions described. The percent rutin trihydrate may be calculated from the following equation:

$$\%\ \text{rutin}\cdot 3H_2O = \frac{A}{ab} \times \frac{V_f}{1000} \times \frac{V_e}{v} \times \frac{100}{w}$$

where A = abc = absorbance of solution (minus blank)

a = A/bc = absorptivity (absorbance referred to unit thickness and unit concentration)
b = cell length, cm.
c = concentration of rutin, grams per liter.
V_f = final volume of rutin-complex solution, ml.
V_e = volume of extract diluted with isoamyl alcohol, ml.
v = volume of aliquot taken, ml.
w = dry weight of sample, grams.

Under the conditions in this procedure, this equation reduces to

$$\%\ \text{rutin}\cdot 3H_2O = \frac{A}{w} \times 10.2$$

Procedures for the Analysis of Rutin Preparations

Porter, Brice, Copley and Couch[578] proposed a spectrophotometric procedure for the analysis of rutin preparations for pigment impurities and rutin content. A method based on this procedure has been incorporated in the National Formulary[742].

Proximate Analysis for Pigments. Weigh out about 200 mg. of the rutin and dissolve in about 20 ml. of absolute ethanol or isopropanol; filter the solution through a small filter paper into a 50-ml. flask, wash the filter paper and funnel with 10-20 ml. of the solvent, and make to a volume of 50 ml. Examine in the visible spectrum for red pigment absorption maximum near 590 mμ and chlorophyll maximum near 655 mμ, using matched 5-cm. cells containing solution and solvent. Measure spectral densities at wavelengths 560, 590, 620, 655 and 690 mμ (or at the red pigment maximum and 30 mμ on each side, and at the chlorophyll maximum and 35 mμ on each side). Calculate absorptivities (specific extinction coefficients) at these wavelengths. Calculate the approximate proportions of red pigment and of chlorophyll in the preparation, using the equations shown below.

$$p = 4\left[a_{590} - \frac{1}{2}\,(a_{560} + a_{620})\right] = \%\ \text{red pigment}$$

$$c = a_{655} - \frac{1}{2}\,(a_{620} + a_{690}) = \%\ \text{chlorophyll}$$

Absorptivity (a) is defined as in the preceding section, i.e., absorbance of solution minus blank, referred to 1 cm. thickness and to concentration of 1 gram per liter for the rutin preparation. Standards set up by the National Formulary IX, limit the amount of each pigment to not more than 0.004%.

Analysis for Rutin and Quercetin. Weigh the sample, by difference, to the nearest 0.02 mg., transferring the sample to a 100-ml. volumetric flask. Add about 5 ml. of absolute ethanol, dissolve with warming if necessary, and finally make the solution to volume with 95% ethanol. Make a 16-fold dilution with 95% ethanol, using 100-ml. volumetric flasks and a 25-ml. pipet. Add 1 ml. of 0.02 N acetic acid to the final dilution before it is made to volume.

Using matched 1-cm. cells containing solution and solvent (also containing acid), measure absorbance (versus solvent) with great care at wavelengths 362.5 and 357.0 mμ. Calculate the absorbance ratio $A_{375.0}/A_{362.5}$. If this ratio is 0.875 ± 0.004 the preparation is free of quercetin, and the percentage of anhydrous rutin in the sample is

$$r = 100a_{362.5}/32.55$$

where $a_{362.5}$ is the absorptivity of the sample at 362.5 mμ and 32.55 is the absorptivity for highly purified rutin under the conditions stated. The value of r is believed reliable to about ±0.5%. The absorptivities* for highly purified rutin[139] and quercetin** are presented in Table VI. The

* The specific extinction coefficient is defined as $K = A/lc$, where A is the absorbance (negative logarithm of the transmittancy), l is the cell length in centimeters, and c is the concentration of solute in grams per liter of solution, in accordance with official nomenclature of the National Formulary IX,[742] p. 767.

** The highly purified quercetin was prepared by W. L. Porter of the Eastern Regional Research Laboratory, according to the following procedure. Refined rutin was hydrolyzed with 0.6% aqueous sulfuric acid and the quercetin obtained was further purified by alternate passage through the pentaacetate[50 a] and the sulfate[50 a] for a total of five preparations of each derivative. The pentaacetate was recrystallized each time from 70% ethanol. The quercetin was recovered after each preparation of the derivatives and analyzed spectrophotometrically. Three passages through the derivatives raised the $K_{375.0}$ to 80.0 which remained unchanged for two additional passages.

TABLE VI

Specific Extinction Coefficients for Rutin and Quercetin

Wave length, mμ	Specific extinction coefficient	
	Rutin	Quercetin
257.7	37.7[a]	75.7[a]
362.5	32.55[a]	72.2
375.0	28.48	80.0[a]
Absorbance ratio[b]	0.875 ± 0.004	1.108 ± 0.020

[a] Maxima.
[b] $A_{375.0}/A_{362.5}$ or $K_{375.0}/K_{362.5}$.

values shown for quercetin are appreciably higher than those reported in the literature summarized by Booth and DeEds.[63a] The previous highest value for $K_{375.0}$ was 77.3, reported by Gage, Douglass and Wender.[243a]

If the density ratio is higher than 0.879, an appreciable amount of quercetin may be present. Calculate the absorptivities at 362.5 and 375.0 mμ, and compute the percentages of anhydrous rutin and quercetin in the preparation by the equations:

$$r = 14.065\ a_{362.5} - 13.181\ a_{375.0} = \%\ \text{rutin}$$

$$q = -5.200\ a_{362.5} + 5.943\ a_{375.0} = \%\ \text{quercetin}$$

The estimated uncertainty is about ± 0.7 both in percentage of rutin and of quercetin, provided errors in absorbance and wavelength are small. Swann[729] suggested that the determination of absorbance at 347.0 and 375.0 mμ gave a more accurate determination of quercetin.

In a more recent method[516] quercetin was separated from rutin by using a short filter paper strip and allowing the rapidly migrating quercetin to travel off the chromatogram into a receiver. The quercetin, thus isolated, is determined spectrophotometrically after reacting with aluminum chloride.

Hörhammer and Hänsel[332] have proposed a method for the determination of quercetin in rutin preparations based on the production of color when zirconium chloride reacts with flavonols.[7] They found that when an alcoholic solution containing the colored zirconium complex of rutin and quercetin is treated with citric acid, the rutin complex was decomposed while that with quercetin remained unchanged and could be measured independently.

Berka and Nesvadba[56] reported that polarography could be used for the individual analysis of rutin and quercetin. However, mixtures of the two compounds gave poorly reproducible curves.

Kakemi, Uno and Iwama[371] proposed a colorimetric method for the determination of rutin. Five ml. of a 60% alcoholic solution containing 0.5 mg. of rutin is treated with 0.3 ml. of a 5% solution of sodium nitrite and 0.3 ml. of a 10% solution of aluminum nitrate. After 3 minutes, 0.5 ml. of a 30% solution of sodium hydroxide is added and the intensity of red color, which is proportional to the concentration of rutin, is measured in a colorimeter.

Determination of Moisture in Rutin. The following method is based on the recent study of Naghski *et al.*[521] This procedure is similar to that used by the National Formulary[742] except that use of a modified weighing bottle

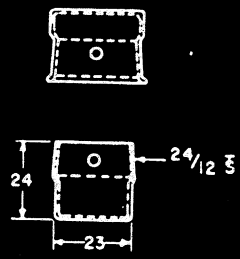

Fig. 13. Special weighing bottle for determining moisture in hygroscopic materials. All dimensions in millimeters.

and continuous sweeping of the oven with dry air are recommended. Dry the special weighing bottle* (Figure 13) in a vacuum oven for one-half hour and cool to room temperature in a desiccator containing an efficient desiccant (anhydrous calcium sulfate or better). Place approximately 0.5 gram of rutin in the tared bottle and weigh to the nearest 0.1 mg. Turn the bottle cap so that the hole is open and place in a vacuum oven at 125°C. With a continuously operating vacuum pump, draw moisture-free air from the drying train** through the oven at approximately 25 ml./min., controlling the air flow with the inlet valve.

* The special weighing bottles (Figure 13) can be made by drilling a 2-mm. hole through the 24/12 ground glass joints of the Parr type weighing bottles. Drilling is done with the cap in place so that the holes in the cap and bottom coincide. When drying a sample in the special bottle the hole is left open to allow the moisture to escape from the sample chamber by diffusion through the opening. Upon removing the bottles from the oven the caps are immediately turned to close the hole. Thus the amount of moist laboratory air coming in contact with the sample after drying is limited to that which would diffuse through the hole during the short time required to remove the bottle from the oven and turn the cap.

** Drying train consisting of a gas wash bottle with fritted glass gas disperser, half filled with concentrated sulfuric acid, followed by a 2-ft. length of 60 mm. O.D. glass tubing containing a 14-in. section of anhydrous calcium sulfate followed by a 6-in. section of phosphorus pentoxide on pumice stone chips. With the drying train and separate outlet and inlet valves, the vacuum chamber can be swept continuously with dry air and the vacuum can be released with dry air at the end of the drying period. To sweep the oven during drying, an air inlet valve is opened slightly so that about 25 ml. of air per minute is drawn through the drying train. The principles involved in this procedure for drying were discussed by Willits.[783]

Determination of Rutin in Tablets

Method I. Porter *et al.*[578] applied the spectrophotometric procedure to the determination of rutin in tablets containing such excipients as lactose, gelatin, calcium sulfate, dicalcium phosphate or other materials having ultraviolet absorption negligible in comparison with that of the rutin present.

Procedure. Weigh to the nearest 0.2 mg. a representative sample consisting of 10 to 20 tablets. Grind in a mortar, place the powder in a weighing bottle, and weigh (by difference) to the nearest 0.2 mg. a sample containing approximately 20 mg. of rutin. Transfer to a 60-ml. centrifuge tube, add 0.5 ml. of water, and stir for a few minutes. Add 25 ml. of 95% ethanol and dissolve the rutin by warming in a water bath. Centrifuge, then wash the insolubles twice by centrifugation, using 25 ml. portions of hot 95% ethanol to which 0.5 ml. of water has been added. Transfer the extract and combined washings to a 100-ml. volumetric flask, cool, and make to volume with 95% ethanol. Dilute 16-fold with 95% ethanol for ultraviolet spectrophotometric examination, adding 1 ml. of 0.02 *N* acetic acid to the final dilution before making to volume.

Measure absorbance at wavelengths 362.5 and 375.0 mμ. Calculate the absorbance ratio $A_{375.0}/A_{362.5}$ and the absorptivities of the solutions at the two wavelengths. (The concentration *c* grams/liter is 10 times the weight of the sample taken after grinding, divided by the dilution factor 16.)

If the absorbance ratio is 0.875 ± 0.004, the sample is essentially free of quercetin. The anhydrous rutin per tablet, in milligrams, is then:

$$r = W\, a_{362.5}/32.55 = \text{mg. anhydrous rutin}$$

where *W* is the average weight of one tablet in mg.

If the absorbance ratio is greater than 0.879, an appreciable amount of quercetin is assumed to be present and the amount of rutin is calculated from the following equation:

$$r = W\,(0.14605\, a_{362.5} - 0.13181\, a_{375.0}) = \text{mg. anhydrous rutin}$$

where *W* has the same meaning as before.

The values for anhydrous rutin so obtained can be converted to rutin·$3H_2O$ by multiplying by the factor 1.0885.

If dicalcium phosphate is used in the excipient, add 15 ml. of 0.02 *N* acetic acid in 95% ethanol to the dry powder followed by 10 ml. of 95% ethanol. Continue as in the procedure described, but do not add more acetic acid to the final dilution.

Method II. Since Method I requires excessive volumes of alcohol for effecting the proper dilutions, there has been a demand for the development of a procedure using other solvents. Turner[755] proposed a modification which employs water for the final dilution. The method was subjected to a collaborative study with satisfactory results.[756] Since the positions of the absorption maxima and the absorptivities for rutin and quercetin in an aqueous solvent are different from those in alcohol, it is considered advisable to present the method in some detail.

Procedure. Determine the average weight per tablet by weighing not less than 20 tablets. Thoroughly powder the weighed tablets and weigh the equivalent of 40 mg. of rutin into a 50-ml. centrifuge tube. Add 0.1 ml. of glacial acetic acid and approximately 15–20 ml. of 95% ethanol. Suspend the powder in the solvent by stirring, and place in a water bath (70°C.) for 10 minutes. Stir occasionally during this extraction period. After heating, remove the stirring rod (wash with 95% ethanol) and centrifuge at approximately 2000 r.p.m. for 10 min. After centrifugation, carefully decant the clear liquid into a 100-ml. vol. flask (use a funnel). While the tube is still inverted in the funnel, wash off the lip with 95% ethanol. Repeat this extraction, starting at "Add 0.1 ml. of glacial acetic acid," twice more. When the contents of the volumetric flask are at room temperature, dilute to 100 ml. with 95% ethanol. Remove any precipitate that may form on cooling or standing by filtration. Transfer 10 ml. of this extract to a 250-ml. vol. flask and dilute to vol. with distilled water. Remove by filtration any precipitate that forms. Determine the absorbance of this aqueous dilution at wavelengths 338.5, 352.5, and 366.5 mμ by means of a spectrophotometer having wavelength errors 0.5 mμ or less. Use 1-cm. absorption cells and employ a distilled water blank.

Calculations:

Using the data obtained, the following calculations are made:

$$a_{352.5} = \frac{A_{352.5}}{bc}$$

where *a* = absorptivity; *A* = absorbance; *b* = cell length in cm; and *c* = concentration of original sample in the final dilution in grams per liter.

$$R_1 = \frac{A_{338.5}}{A_{352.5}} = \text{ratio of absorbance at 338.5 and 352.5 m}\mu$$

$$R_2 = \frac{A_{366.5}}{A_{352.5}} = \text{ratio of absorbance at 366.5 and 352.5 m}\mu$$

If R_1 equals 0.909 ± .009 and R_2 equals 0.846 ± .009, the extracted material can be considered pure rutin and the weight (in mg.) per tablet can be calculated by means of the following equation:

$$\text{mg. Rutin}\cdot 3H_2O/\text{tablet} = \frac{a_{352.5}}{26.3} \times \text{av. wt./tablet (mg.)}$$

Since pharmaceutical rutin[742] may contain up to 5% quercetin an "acceptable" range is suggested for the above ratios: for R_1 a range of 0.890 to 0.918; for R_2 a range of 0.837 to 0.878. If the sample falls within these ranges the above equation may be used for calculating the rutin content. An increase in R_2 above this limit with a simultaneous decrease below the R_1 limit indicates that the sample contains more than 5% quercetin. In such cases the amount of rutin may be calculated from the following equation:

$$\text{mg. Rutin}\cdot 3H_20/\text{tablet} = (0.1475a_{352.5} - 0.1292a_{366.5}) \times \text{av. wt./tablet (mg.)}$$

An increase or decrease beyond the limits of both ratios indicates an interfering absorption which invalidates the analysis.

An increase of R_1 beyond its limit while R_2 remains within its range indicates an interfering absorption at 338.5 millimicrons which diminishes so as to be ineffective at the R_2 wavelength. Under these conditions the correctness of the observed value at 352.5 millimicrons is accepted because any elevation of the 352.5 millimicron reading would lower R_2.

Method III. A method, based on the production of color when rutin reacts with aluminum chloride is proposed by Dechene[170] for the determination of rutin in tablets. This method offers the advantage that the absorption maximum of the reaction product is in the visible region of the spectrum (415 mμ).

Procedure: The average weight of one tablet was determined from the weight of 15 to 20 tablets and 5 to 10 tablets were reduced to a fine powder. A weight of powder, calculated to contain 15–20 mg. of rutin, was placed in a micro-Soxhlet apparatus and extracted for eight to ten hours with ethyl alcohol (denatured). The alcoholic extract was transferred to a 50-ml. volumetric flask, made to volume with the alcohol, and 15 ml. of this solution was transferred to a 50-ml. volumetric flask and made to volume with alcohol.

Duplicate 1-ml. aliquots of the solution were transferred to test tubes, and alcohol was added to bring the volume to 5 ml. To each tube was added 3 ml. of 0.1 *M* aluminum chloride solution and 5 ml. of 1.0 *M* potassium acetate solution. After standing forty minutes, the transmittance of the yellow color was determined at a wavelength of 415 mμ. The quantity of rutin is determined by reference to a previously prepared graph using solution of known rutin content.

Determination of Rutin in Urine

A method for the determination of rutin in urine was developed by Porter *et al.*[579] To 2 ml. of urine is added 3 ml. of 0.1 *N* $AlCl_3$ and 0.5 ml. of NH_4OH (1:3). This is diluted to 10 ml., centrifuged, and the supernatant liquid decanted. The gel is washed twice with water to which is added 1 drop of the dilute NH_4OH, and all washings discarded. Finally, the gel in the tubes is dissolved by adding 0.1 ml. of glacial acetic acid, dispersing the precipitate, and letting stand over night. Then 10 ml. of 1 *N* potassium acetate is added and the mixture is transferred to a 50-ml. volumetric flask and made up to volume with water. After a period of at least 30 minutes, but not more than 2 hours, the absorbance is determined at 413–416 mμ in a 5-cm. cell against a reagent blank carried through the same procedure. The amount of rutin is determined by reference to a previously prepared graph[579] using solutions of known rutin content.

Production of Rutin

For use in clinical work rutin was first supplied by the Eastern Regional Research Laboratory, Bureau of Agricultural and Industrial Chemistry, United States Department of Agriculture. It was prepared from tobacco by a percolation procedure using ethyl alcohol as the solvent.[130] However, the low yield (0.3–0.5%), from an expensive raw material, made it essential to find a more economical source. Investigation of many plants revealed buckwheat as the most promising domestic supply,[137] since it was found to contain 3 to 6% rutin on a dry weight basis. Of the several species that are commonly used for grain, the Tartary (*Fagopyrum tataricum*) was found to be superior to the Japanese or Silver Hull (*Fagopyrum esculentum*) as a source of rutin.[140] The major portion of the rutin occurs in the leaves and blossoms and reaches a maximum in 35 to 45 days. The rutin can be extracted either from the fresh plant[132,133] or from a meal prepared by artificial drying of the plant.[134,207,208,398,513,520,573]

Recently two other source materials have become available for rutin manufacture; dried flower buds of *Sophora japonica*[138] from the Orient and the leaves of several Eucalyptus species (*Eucalyptus macrorrhyncha* and *Eucalyptus youmani*)[400,616] from Australia. Sophora flower buds contain 12–16% rutin and Eucalyptus leaves about 6–11%. These source materials have almost completely displaced buckwheat in this country chiefly be-

cause the American dehydrators failed to produce high grade buckwheat leaf meals;[513] only whole buckwheat meals were produced. These did not contain more than 2.5 percent rutin. It is possible to prepare a domestic buckwheat product containing 5 to 8% rutin[513] which manufacturers have indicated would compete favorably with foreign sources.

Preparation of Dried Buckwheat Leaf Meal

Rutin can be extracted from either the fresh green immature plant or from a meal prepared by drying and grinding the green plant.[398] Fresh plants have the disadvantage of being available only during the limited

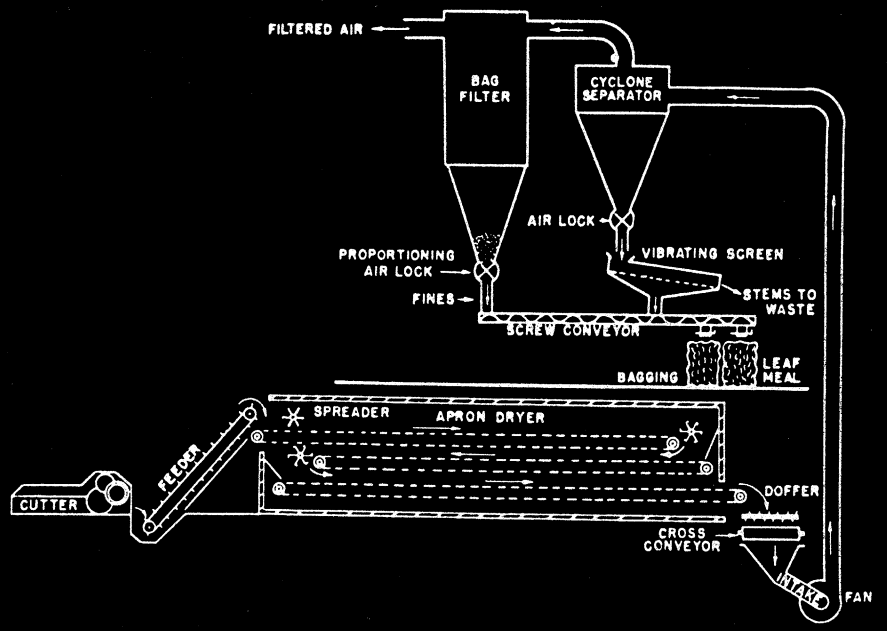

Fig. 14. Flow diagram for preparation of buckwheat leaf meal in a belt dryer.

growing season, whereas the dried product can be stored for year-round production. Early work[137] showed, however, that buckwheat had to be dried carefully to prevent excessive destruction of the rutin.

Fractional Drying. The finding that the major portion of the rutin was located in the leaves and blossoms of the immature buckwheat[520] suggested that rutin could be concentrated by the preparation of a leaf meal. Eskew and coworkers worked out in detail a procedure for producing a dried leaf meal, using either a belt[207,208] (Figure 14) or a direct fired rotary drier[573] (Figure 15).

The recommended procedure is to dry as quickly as possible and carry the drying only far enough to embrittle the leaves and flowers; the stems, being much thicker, remain moist and tough. The plants in this condition are subjected to mechanical action, which breaks the brittle leaves and flowers

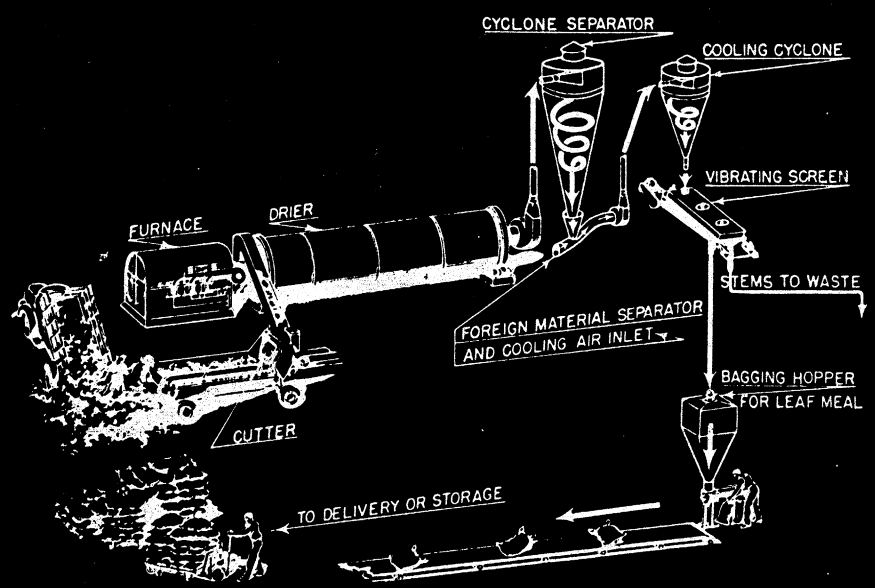

Fig. 15. Production of buckwheat leaf meal in a rotary alfalfa dryer.

away from the stems and crushes them into fragments. This is accomplished by directing the fractionally dried buckwheat into a fan, which, acting somewhat as a hammer mill, strips the friable leaves from the limp stems. The fan also blows the material to a cyclone collector. From there it falls to a vibrating screen, which separates the stems from the leaf fragments. A bag filter is provided on the cyclone exhaust to recover rutin-rich fines. The two fractions are illustrated in Figure 16.

The drying process has to be carried out under strictly controlled conditions to prevent excessive loss of rutin. Even under optimum conditions for fractional drying, 25 to 35% of the rutin is lost; if the buckwheat is totally dried, the destruction is somewhat greater. The degree of rutin destruction depends on the type of drier, the temperature and the variety of buckwheat used.

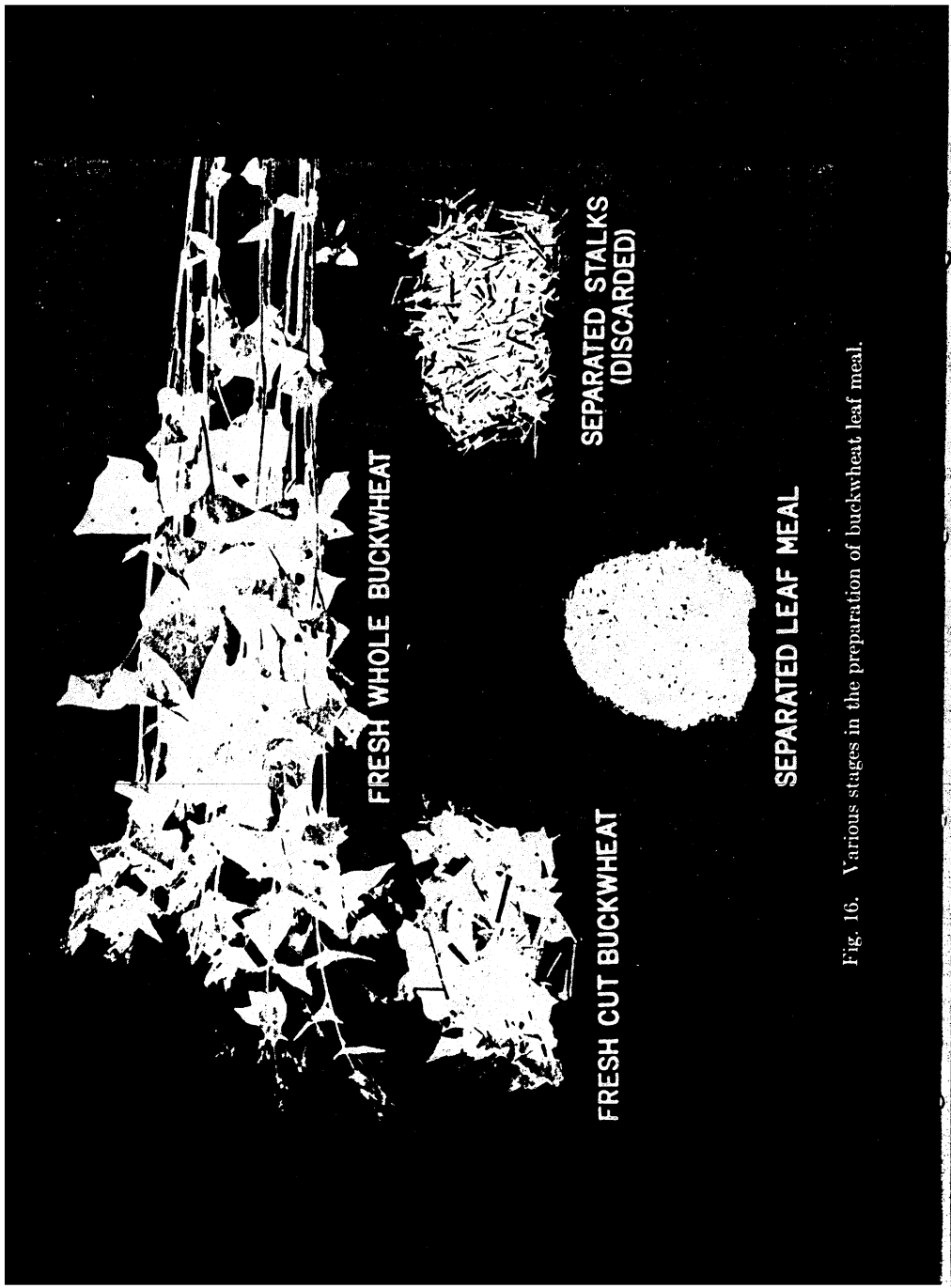

Fig. 16. Various stages in the preparation of buckwheat leaf meal.

Although it is possible to hand-pick a leaf and blossom fraction which would result in about a 1.8-fold enrichment in rutin content[520], this separation has not been done mechanically on a commercial scale. An evaluation of a number of leaf meals produced at this laboratory during the pilot plant study of drying conditions showed that a 1.5-fold enrichment could be produced over that which could be obtained when whole meals were prepared. This enrichment was more than enough to compensate for losses encountered during drying, and the leaf meals were richer in rutin than the starting fresh plant.[520] Since in these studies only 60% of the stem material was discarded, there is a possibility that removing a greater proportion of the stems[573] would produce even a greater degree of enrichment.

Field drying as an adjunct to artificial drying has been investigated.[520,573] Under favorable conditions as much as one-third of the water could be removed from normal buckwheat or one-half from unusually lush buckwheat without reducing the over-all recovery of rutin, provided that wilting was not carried far enough to reduce the moisture content below 78%. Wilting greatly increases the capacity of the drier and decreases the cost of fuel. Under unfavorable weather conditions when wilting was slow, however, an appreciable loss of rutin occurred.

Relation of Buckwheat Variety to Rutin Content. Buckwheat first used for production of rutin was the Japanese (*Fagopyrum esculentum*),[137] commonly grown for grain. Later work,[140] however, showed that the little known Tartary (*F. tataricum*) was superior in several respects. It was 45 to 80% richer in rutin, had a higher proportion of leaf, and yielded greater quantities of leaf per acre. Furthermore, Tartary is more frost resistant and so can be planted earlier in the spring. In addition to these cultural advantages, Tartary buckwheat is better suited for dehydrating. It can be dried at higher temperatures and with less critical control than the Japanese.[208] Work with three additional varieties of buckwheat—Silver Hull, Tartary Tetrapolid and Emarginatum—indicated that, for rutin production, Tartary is also better than the Silver Hull and Emarginatum.[776] The Tartary Tetrapolid[632] is similar to the Tartary in rutin content and yields and so has potentialities for rutin production; however, seed is not available in commercial quantities.

Effect of Age of Plant and Time of Planting on Rutin Content. Figure 17 shows the effects of age and time of planting on the rutin content of Tartary and Japanese buckwheat. It can be seen that buckwheat plants reach the peak of rutin content at the time of blooming which is about 25 to 35 days after emergence, depending on the species. The rutin content of all buckwheat decreases after seeds form. The decrease in rutin content of Tartary buckwheat is not so rapid or so great as that of the Japanese. Tartary both flowers and sets a full crop of seed more slowly.

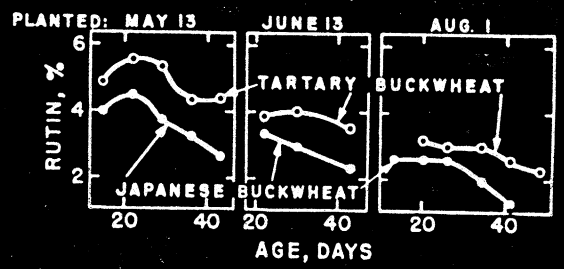

Fig. 17. Effects of age of plant and time of planting on the rutin contents of Japanese and Tartary buckwheats (grown at Lancaster County Tobacco Experiment Station, 1946).

It does not mature so rapidly but grows continuously, maintaining a considerable portion of the plant as young immature tissue, which is always richer in rutin.

Buckwheat is ordinarily seeded for grain production in late June or early July. Good yields of rutin were obtained in Pennsylvania by seeding on May 13 (Figure 17); the rate of growth was slower in early plantings but

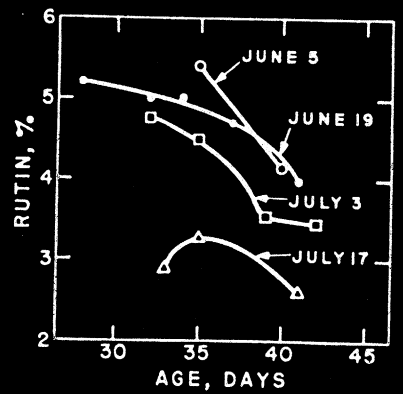

Fig. 18. Effect of date of planting on rutin content of Tartary buckwheat.

the percentage of rutin was higher. Seeding on June 13 and August 1 gave larger plants, but the percentage of rutin was progressively lower.

In 1948, four large-scale plantings of Tartary buckwheat were made on Lansdale silt loam, Montgomery County, Pennsylvania, to supply material for drying studies with a portable alfalfa drier.[573] Figure 18 shows the rutin content of buckwheat harvested from these four plantings. Here again the buckwheat from the earliest plantings had the highest rutin content, and the rutin decreased progressively as the season advanced. Since in most cases the rutin content of leaf meals prepared by fractional drying is only slightly higher than that of the fresh plant, to produce leaf meal of high rutin content, Tartary buckwheat must be harvested at the peak of its rutin content. The best time will vary with the season, but it will be about 4 to 6 weeks after seeding.

It is doubtful that a buckwheat meal sufficiently rich in rutin could be produced from buckwheat planted later than June. The date of planting will depend somewhat on the geographic location. Where the climate is cooler and growth is slower, planting may be delayed somewhat.

Extraction

Krewson and Couch[398] have determined the optimum conditions for the extraction and preparation of rutin from buckwheat. Chainey[91] offers a modification to the procedure which gives an alternative method for removal of fats during the extraction. A diagrammatic sketch of this process is shown in Figure 19. Either fresh green buckwheat or dried buckwheat meal is placed in a suitable extractor and covered with isopropyl alcohol (approximately 0.4 gallon per pound of 80–85 percent strength by volume for green plant; approximately 1.5 gallon per pound of 70–85 percent strength for dried buckwheat meal). The mixture is heated to boiling, and following a short interval (10 minutes) the extract is pumped to an evaporator. Extraction of rutin is complete, no agitation is necessary, and the marc need only be washed several times with hot solvent to remove rutin dissolved in entrained solvent. The extract and washings are concentrated in an evaporator until the solvent is removed (to about one-tenth original volume). When operating under reduced pressure, sufficient boiling water should be added before or during evaporation to about double the water contributed by the dilute solvent used in the extraction. This is to prevent precipitation of rutin from the super-saturated concentrate. When operating under atmospheric pressure the temperature during the evaporation is high enough to prevent rutin precipitation, and boiling water (2–3 volumes) is added after evaporation of the solvent is complete.

The boiling concentrate is then strained through a filter, consisting of a glass wool mat supported on a coarse wire screen, into a heated holding tank in order to remove the bulk of the fat.[131] Final traces of fat are removed by filtering the boiling solution through heavy paper filter pads. The concentrate is then cooled to effect rapid crystallization of crude rutin. After 1 to 2 hours, the cold crude rutin is filtered off on canvas or on a hard

filter (preferably rayon paper) and either refined immediately or stored dry for subsequent purification.

In the preparation of rutin from Sophora and Eucalyptus, or other high rutin content plant materials, it is possible to extract the flavonol with boiling water. The dry ground plant material is exhaustively extracted with boiling water, the hot water extracts are polished through efficient filters, and the filtered extract cooled rapidly to effect rutin crystallization.

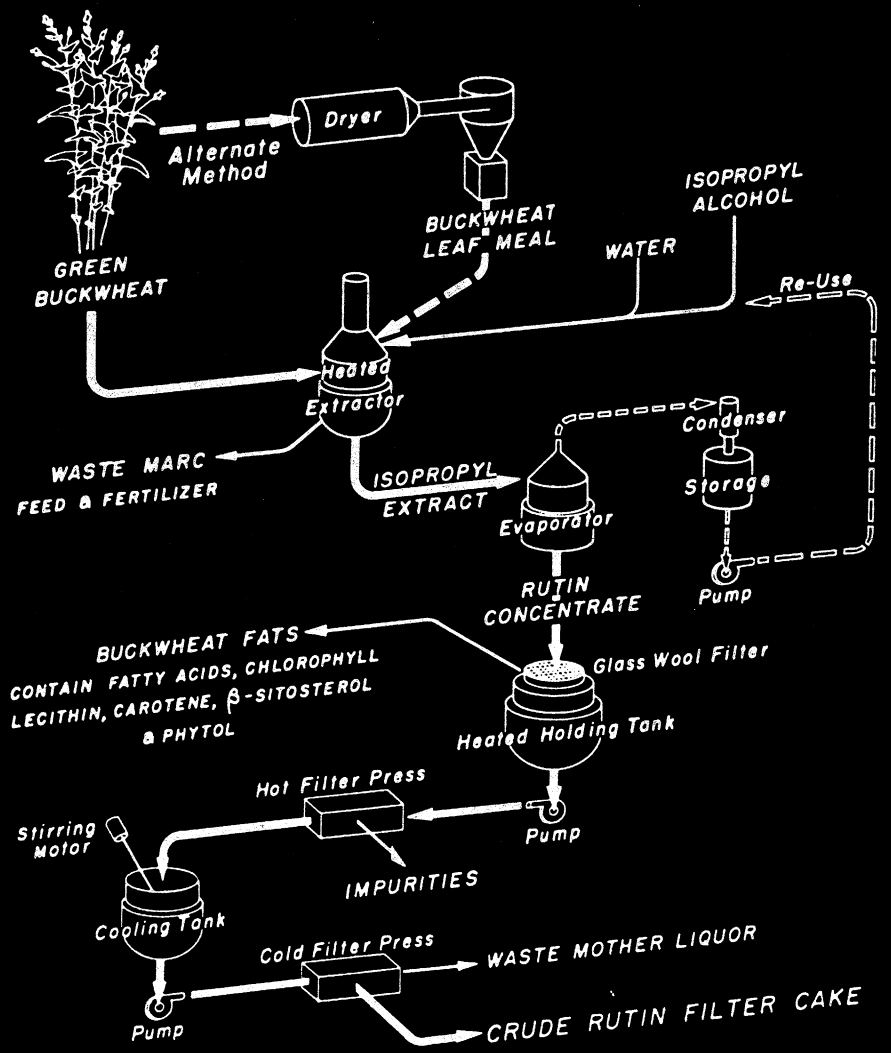

Fig. 19. Diagrammatic scheme of process for the production of rutin from buckwheat.

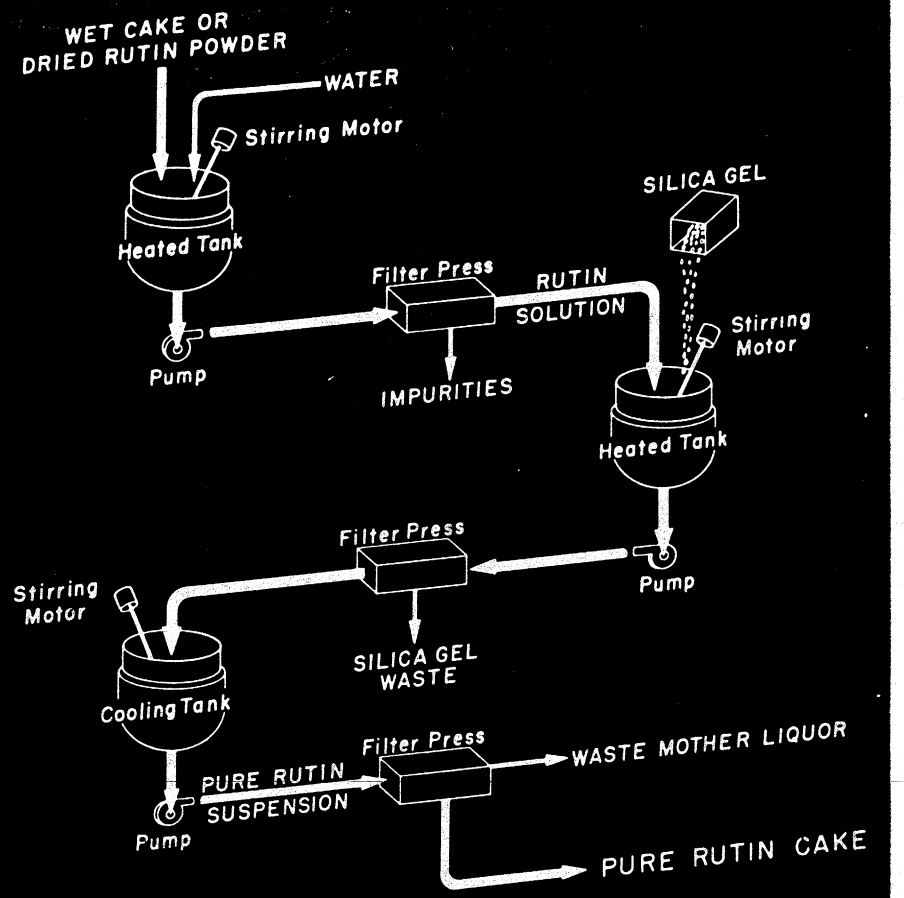

Fig. 20. Diagrammatic scheme of process for refining rutin.

Purification of Rutin

Recrystallization. Refining of crude rutin prepared from buckwheat is illustrated in Figure 20. Crude rutin is dissolved in boiling water (about 18–20 gallons to the pound of dried crude rutin), and filtered to remove finely divided insoluble material.[132,133,398] Purification of buckwheat rutin requires treating the boiling filtered solution with silica gel (usually about $\frac{1}{2}$ pound of a 28 to 200 mesh silica gel per pound of dried crude rutin) to remove so-called "red pigment" impurity.*[135] After filtration the rutin solution is cooled rapidly to effect crystallization and the crystals removed as soon as possible. The quick cooling of the rutin solution to a low tem-

* Rutin prepared from other plant sources which do not contain this impurity, do not require treatment with silica gel.

perature gives rise not to small crystals as in the usual rapid crystallization procedures, but to a solid material consisting of flocks of crystals larger than those obtained by crystallization at room temperature. According to the generally accepted theory relating to purification by crystallization procedure, it is considered desirable to produce fine crystals since these have a tendency to occlude smaller amounts of impurities than larger crystals. In the case of rutin, the reverse appears to be true. Also, it appears that in the crystallization of crude rutin, contamination with extraneous products dissolved or dispersed in the solution is promoted by prolonged contact of the solid rutin particles with the mother liquor. Therefore, it is desirable that the crystallized rutin be removed from the mother liquor as soon after completion of crystallization as possible.[395] The product is then dried to constant weight at 110°C.

Removal of Alcohol Insolubles. The rutin prepared as outlined above may still contain excessive amounts of alcohol insoluble impurities. These can be removed by treating the rutin with absolute isopropyl alcohol. The powdered rutin, thoroughly dried at 110°C., is dissolved in boiling isopropyl alcohol of 98–99% strength at the rate of 1 pound of rutin per 1.75 gallons of alcohol. The solution is cooled to room temperature and filtered through heavy asbestos pads. The rutin may be recovered by crystallization after diluting the alcohol filtrate with 9 to 10 volumes of water. Economy of solvent can be effected by adding the filtrate to boiling water and distilling off the alcohol for re-use.

Removal of Metal Impurities. Rutin that has been prepared or has come in contact with equipment made of iron, copper, or aluminum, becomes contaminated with complexes of these metals. Since these complexes are water soluble compounds that tend to form mixed crystals with rutin and are not removed by adsorbing agents, they cannot be eliminated by the usual purification methods. The presence of these impurities has a detrimental effect on rutin, causing discoloration and decomposition of rutin preparations, especially on exposure to light, and consequently their elimination is essential to the production of rutin preparations intended for medicinal use. The presence of appreciable quantities of these impurities also causes considerable losses in the course of usual purification treatment; in such instances as much as 10 per cent of the rutin present fails to crystallize out on cooling and cannot be obtained even after extensive concentration of mother liquors.[403] Purification of such contaminated rutin is accomplished by dissolving the product in boiling water (one pound in 20 gallons) and acidifying the solution to pH 2.5–2.9 by addition of dilute mineral acids such as hydrochloric, sulfuric or phosphoric. In the case of contamination with iron the solution will change from green to pale yellow. For copper contamination it is necessary only to adjust to a pH less than 4. On standing at room temperature rutin will separate from the solution in the form of a pale yellow crystalline precipitate which is removed by filtration, washed with cold water, and dried to constant weight at 110°C. Although the process is operable at a pH of less than 2.5, hydrolysis of rutin is likely to occur and rutin solutions below this pH should not be held at elevated temperatures.

Preparation of Highly Purified Rutin. The fact reported previously that rutin forms solvates with various organic solvents led to a new method for the preparation of highly purified rutin.[139,401] Buckwheat rutin prepared by the present accepted procedure[398] (N.F. quality) contains small amounts of quercetin and other related flavonoids. These minor flavonoids interfered in the spectrophotometric analysis and values greater than 100% were obtained for the sum of rutin and quercetin. Repeated recrystallizations from water or aqueous solvents as the hydrate did not reduce significantly the quantities of these impurities. However, when rutin crystallized as the solvate the major portion of the impurities remained in the organic solvent solution. The effect on the purity of rutin when samples were crystallized from technical absolute methanol, ethanol, n-propanol and acetone is shown in Table VII.

TABLE VII

Effect of Recrystallization from Organic Solvents on Purity of Rutin[a]

Recrystallized from	Spectrophotometric analysis	
	Rutin, %	Quercetin, %
Methanol	98.3	1.4
Ethanol	100.0	0.0
n-Propanol	100.6	1.3
Acetone	100.5	0.0

[a] Original rutin analyzed 97.3% and 4.3% quercetin.

Of the four solvents used in purification of rutin, ethanol and acetone gave the purest products. The yields were good since the second crop of rutin obtained from mother liquors may be reprocessed. Ethanol is the most practical of these two to use because the solubility of rutin is greater in ethanol than in acetone and the amount of moisture present in the solvent is not as critical. The solubility of rutin in absolute ethanol is greater than 10% but more concentrated solutions were difficult to handle especially after the laboratory became seeded with alcoholate. When the laboratory did become seeded, the alcoholate precipitated from absolute alcohol containing more than 10% rutin, even while the solution was boiling under reflux.

Purification of rutin by alcoholate procedure is now in operation on a commercial scale for the production of highly purified rutin required to make soluble preparations of rutin for parenteral use. Rutin prepared through the alcoholate procedure was the only rutin we have obtained which did not discolor when exposed to light; rutin recrystallized 15 times from aqueous solvents became discolored.

Procedure. The procedure for this method of purification is as follows: An approximately 10 percent solution of rutin is prepared by dissolving *thoroughly dried* (at 110°C.) powdered rutin in boiling absolute ethanol (denatured containing 95% absolute ethanol and 5% absolute methanol is satisfactory). The boiling solution is filtered preferably through a heavy paper and asbestos filter pad and washed with absolute alcohol. Crystallization usually takes place immediately on cooling of the filtrate especially when the process is in regular operation and the laboratory or plant atmosphere contains the alcoholate seed (in initial operation it may be necessary to seed the solution to effect crystallization). The rutin-alcoholate crystals are filtered off after 2 hours standing at room temperature, then slurried with a small quantity of absolute ethanol, filtered by suction and the filter cake added to about 10 quarts of boiling water for hydrate formation.

General Physiology Involved in Vascular Fault

The blood vascular system is usually considered to consist of the heart, aorta and large arteries grading down to the small arterioles, the capillaries, and then the venules grading up to the large veins and so back to the heart. By way of the vascular system, the tissue cells receive oxygen and substances needed for their metabolism. Moreover, waste substances are removed. Oxygen, being transported by the hemoglobin of the red cells, may be thought to diffuse directly from the capillaries. However, water and most dissolved substances, including a small part of the plasma protein, actually pass out of the capillaries into the tissues. From the tissues, water and excretory metabolites pass either back into the capillaries or enter lymphatic radicles, through which they pass to larger and larger lymphatic vessels until finally they enter the venous system. Under conditions of rest, probably a considerable part of the water and dissolved substances leave the tissue to re-enter the capillaries. However, under conditions of greater circulatory activity, and greater diffusion into the tissue, the quantity re-entering the capillaries from the tissue probably increases very little, if any, and the greater amount of increased fluid enters the lymphatic system.

The perivascular tissue consists of fixed cells of varying degrees of specialization, embedded in a more or less fluid, gelatinous matrix which is referred to as the mesenchymal ground substance. The three possible routes for fluid circulation may therefore be considered somewhat as follows:

Route A: Heart to arteries to capillaries to veins to heart.
Route B: Heart to arteries to capillaries to mesenchymal ground substance to capillaries to veins to heart.
Route C: Heart to arteries to capillaries to mesenchymal ground substance to lymphatics to veins to heart.

Route A may be sufficient to supply oxygen and nutrients to the most adjacent endothelial lining cells, but probably only oxygen to the other tissues. In routes B and C the fluid leaving the capillaries to enter the mesenchymal ground substance passes between, not through, the endothelial cells of the capillary wall; i.e., it traverses the intercellular cement sub-

stance. In route B it also traverses this intercellular cement substance on returning to the capillaries; and in route C the intercellular cement substance of the endothelial cells of the lymphatic vessel. However, from our interpretation of published data, any abnormality of intercellular cement substance through which fluid *returns* to vessels is not recognized as being of any importance. On the other hand, abnormality of the intercellular cement substance through which fluid leaves the capillary may be recognized as such if it results in red cells passing out of the capillary and becoming evident in the pericapillary tissue.

Circulation by route A is quite rapid, the entire circuit being completed in a few minutes. While time required for routes B and C is not known, it is undoubtedly much longer. In life, the mesenchymal ground substance is gelatinous and probably varies considerably in different tissues. In fixed specimens its coagulated remains appear as fibers and fibrils, which probably represent structures not so well defined during life. Mesenchymal ground substance which contains collagen is sometimes referred to as collagenous ground substance, and its pathologic states are spoken of as collagen diseases; although it is probably inherent in its nature to escape exact morphologic classification. The electron microscope may aid considerably in the understanding of this substance. This ground substance can bind and release water, and substances contained therein, depending upon the needs of the fixed tissue cells or according to a variety of stimuli not now entirely understood. This ground substance may be large in amount, as in the subcutaneous tissue, or small, as it must be between the glomerular capillary and the capsule of Bowman. It may readily admit fluid from the capillary, which would decrease the gradient of pressure from within the capillary to the tissue outside and hence theoretically predispose to capillary hemorrhage. Such hemorrhage might be absorbed rapidly or slowly, depending upon the speed with which fluid was released by the mesenchymal ground substance to enter the lymphatics. On the other hand, if the pressure in the tissue outside the capillary is relatively high (the reverse of above) the capillary wall will be somewhat supported and the development of capillary hemorrhage will be opposed.

Functionally, therefore, the intercellular cement substance and the mesenchymal ground substance form a single unit, and it is not possible to say that what affects one does not affect the other. However, it may be suggested that an agent which either causes or prevents the development of capillary hemorrhage has an action on the intercellular cement substance, and this effect cannot be assumed if there is no relation to capillary hemorrhage. Among substances now thought capable of affecting the intercellular cement substance or the mesenchymal ground substance, or both, are rutin and other flavonoids, natural adrenal cortical hormones including the synthetic adrenal cortical hormone, desoxycorticosterone, X-ray, hyaluronidase, probably a pituitary hormone, vitamin A, vitamin E and certain amino acids.

In addition to the above, it is suggested that under certain abnormal conditions something is absorbed from the mesenchymal ground substance to which the body reacts by the production of antibodies. These antibodies, in turn, may act with sufficient specificity on the tissue from which their exciters originally arose to further and characteristically damage it.[700] Substances capable of blocking antibody formation may be of value in addition to the factors listed.

Abnormality of the intercellular cement substance may lead to the following pathologic states: Increased fluid, which may contain an increased amount of protein, may pass into the mesenchymal ground substance; red cells may escape either through rupture or by diapedesis. As a result of these two conditions abnormalities may be produced in the mesenchymal ground substance, or a clot may be formed at the point of rupture and give rise to an extending thromboembolic process.

Abnormality of the mesenchymal ground substance may lead to the following pathologic states: Its failure to adequately bind fluid, and hence substances contained therein, may lead to death and destruction of fixed cells. If this occurs in the wall of an artery, it may lead to rupture through that wall, or if in the retina, to death of the retinal nerve cells. It may lead to antibody formation (which may contribute to its later destruction) by releasing antigenic protein molecules, from itself or from fixed tissue cells, into the general circulation. It may bind excessive fluid and produce actual or latent tissue edema causing disturbances in tissue function; perhaps this is secondary to changes in sodium and potassium transport. A sustained pathologic process involving the mesenchymal ground substance may terminate with this substance becoming less gelatinous and more fibrillar, with an absolute decrease in bulk.

Factors Affecting the Integrity of the Capillary Wall and Extracapillary Fluid Transfer

The role of rutin and other flavonoids in affecting the integrity of the capillary wall and extra-capillary fluid transport will be considered in greater detail in subsequent chapters. Here we shall attempt to summarize the effects of the other factors.

The Adrenal and Adrenal Hormones. In the absence of the adrenals, sodium is lost through the kidneys and eventually death results with hypotension. This sodium loss can be prevented by the administration of

adrenal cortical extracts, the medulla apparently not being essential to life. A synthetic hormone, desoxycorticosterone acetate (DOCA), also has this salt-retaining life-preserving function in the adrenalectomized animal, and apparently corresponds to that part of the natural adrenal cortical hormone which is secreted by the zona glomerulosa. It was Selye[671] who showed that this hormone, given in large doses, produced mesenchymal proliferation along with periarteritis, nephrosclerosis and arthritis. It has thus been possible to compare the effects of extracts of the whole adrenal cortex with the effect of a single one of its hormones. More recently the availability of other adrenal cortical hormones as prepared by Hench and his associates[315] has permitted more exact comparisons to be made.

Seifter, Baeder and Dervinis[670] suspended strips of urinary bladder in an osmometer and measured the passage through it of fluid and electrolyte. Permeability was increased by adding either hyaluronidase or desoxycorticosterone to one side of the membrane, decreased by adding whole adrenal cortical extract. Later, using rabbits, Seifter, Baeder and Begany[668,669] were able to show that absorption of phenolsulphonphthalein from the synovial joint cavity of the knee was increased if hyaluronidase was added to the injected material, or if desoxycorticosterone was given parenterally. Absorption was decreased by the administration of either whole adrenal cortical extract, cortisone, or adrenocorticotropic hormone. Absorption was measured both by the rate of the excretion of the dye in the urine, and by measuring the amount left in the joint cavity on aspiration after a certain time. Adrenal cortical extract antagonized the effect of both hyaluronidase and of desoxycorticosterone. The authors suggest that "normal permeability of the synovial membrane is in part controlled by the balance between adrenal steroids of the DOCA type and of the Compound E type."

It is interesting to note that in this study, using rabbits, the administration of the adrenal cortical stimulating extract of the anterior lobe of the pituitary (ACTH) results primarily in an increase in secretion which, in action, most resembled Compound E. In the studies, by other workers in rats, ACTH seems to primarily result in secretion of hormone of the desoxycorticosterone (DOCA) type. It is possible that such species differences may exist, and it is not certain which corresponds with the human-type reaction.

Taubenhaus and Amromin[739] have shown a similar antagonism between "desoxycorticosterone (which) if injected into intact animals prior to and during the turpentine abscess formation had a profoundly stimulatory effect (upon) fibroblasts and. . .ground substance, (and) cortisone (which) had an inhibitory effect. . ." They also found that growth hormone of the anterior pituitary stimulated granulation tissue, but that this effect was abolished by adrenalectomy.

Cope, Brenizer and Polderman[119] collected lymph from the cervical trunk of normal and adrenalectomized dogs under local anesthesia. The protein content of the lymph of the adrenalectomized animals was significantly higher, a finding which they credited to an increased capillary permeability. Cope and Moore[120] studied capillary permeability in dogs by injecting radioactive colloids, and found an increased permeability following a burn. This was not affected by giving adrenal cortical extract intravenously.

Rigdon[609,610] gave adrenalin (*l*-epinephrine) intravenously or intradermally to rabbits before applying xylol to their skin. Subsequently, "trypan blue, when given intravenously, fail(ed) to localize and to concentrate in areas. . .where adrenalin (was) injected intradermally. . .although the area becomes reddish brown after 20–30 minutes." However, when the epinephrine was given intravenously, the localization of trypan blue in the area of xylol irritation was not prevented, a difference which the author attributes to a lower concentration of epinephrine in tissue after the intravenous injection. Other studies, using *l*-epinephrine and *l*-arterenol,[105] also suggest that the secretion of adrenal medulla may have an effect on the capillary wall. The situation is further complicated by the fact that the administration of epinephrine[745] will, through the pituitary, cause stimulation of the adrenal cortex. This would not explain Rigdon's results, since he got no inhibition of increased permeability when the epinephrine was given intravenously. There is the possibility that in his studies, epinephrine interfered with the circulation by producing a local closure of vessels and thus may have masked rather than prevented the increase in permeability. Describing the injected area as reddish brown (not blanched white) does not support such an explanation.

Vitamin C. The relation of vitamin C deficiency to increased capillary fragility will be considered in more detail later. However, it may be stated here that vitamin C probably has no direct effect on capillary fragility, but that by producing atony on the venous side of the minute vessel bed and probably by some effect on the perivascular tissue in lessening support of the minute vessel bed, its lack results in the occurrence of hemorrhagic lesions, often of considerable size, resulting from minimal trauma. Vitamin C is also an important constituent of the adrenal gland, and Schaffenburg, Masson and Corcoran[641] have compared the syndrome of vitamin C with that due to adrenal deficiency. There are many points in common, and it is suggested that death, when it occurs in scurvy, is actually due to adrenal failure. It has long been recognized that death in the scorbutic patient cannot always be explained on the basis of hemorrhage. Cortisone helps the scorbutic guinea pigs (but does not prevent hemorrhage) while DOCA makes the animal worse.

Hyaluronidase. The role of hyaluronidase in vascular physiology has been summarized by Meyer and Ragan[496] and is here briefly reviewed: Connective tissue consists of fibrillar material and interfibrillar substances. The latter consists of an amorphous and viscous ground substance, and the cement substance proper. While the chemical nature of these substances is not completely known, they do contain two compounds, hyaluronic acid, and chondroitin-sulfuric acid, and may be acted upon with resulting increase in fluidity, by an enzyme hyaluronidase which may be derived from various sources. "Chondroitin sulfate occurs in the intima of arteries. This layer may even continue to the precapillaries and capillaries. That this zone does exist in the capillary wall is suggested by the increase in capillary permeability caused by hyaluronidase injected into the connective tissue, whereas intravenous injection had no effect."[92] The highest concentration of hyaluronic acid in the mammalian body is in synovial fluid and skin; next highest is in the vitreous humor. Hyaluronidase may be produced by certain pathogenic organisms, and by certain malignant tumors. Hyaluronidase-inhibiting substances may also appear in the body, either spontaneously or as the result of some disease process. The evidence is quite clear that hyaluronidase will expedite the passage of fluid through the mesnchymal ground substance once that fluid has reached an extravascular position; it is much less clear that it has any direct effect on passage of fluid through the capillary wall.

Vitamin E. Although it is not our purpose to review the involved and highly controversial subject of the role of vitamin E in vascular physiology, we would like to point out that this vitamin is thought by some to have an effect on the mesenchymal ground substance,[84,330] and by a few to have also an effect in lessening increased capillary fragility.[688,692]

Vitamin A. Mayer and Krehl[491,492] showed that when rats were given a diet deficient in vitamin A they developed bleeding of the lacrimal glands, red and swollen gums, and swollen joints. Since this resembled a vitamin C deficiency, vitamin C was added to the vitamin A deficient diet, and the scorbutic symptoms disappeared.

Burn, Orten and Smith[85] maintained rats on a diet with minimal vitamin A and found histologic changes in their molars suggesting scurvy. Boyer, and co-workers[69] gave calves a diet deficient in vitamin A and found a drop in ascorbic acid content of both the blood and cerebrospinal fluids. The drop in vitamin C content of the cerebrospinal fluid was inversely proportional to the rise in cerebrospinal fluid pressure. Jonsson, Obel and Sjöberg[368] found dental changes after vitamin A deficiency in the rat similar to those described by Burn *et al.*,[85] and concluded that, in vitamin A deficiency the rat loses the ability to synthesize ascorbic acid.

However, while animal experiments of this type may suggest some relationship between certain hemorrhagic states and vitamin A deficiency, so far as we know no such relationship has been demonstrated in man.

Various Possible Interrelations. In previous publications[281,291] one of us (JQG) has reported studies on the rate of lymph flow in the skin of rats given posterior pituitary hormone which produced antidiuresis, and in humans thought to have an excess of this hormone, on the basis of bioassay tests in animals. It was thought that increased pituitary activity led to an increase in movement of lymph through the skin, and this was in accord with other studies reported prior to the recent upsurge in the knowledge of adrenal function. Since no adrenal studies were made in the papers just mentioned[281,291] and since the pituitary is known to stimulate the adrenal, it is quite possible that the findings at that time ascribed to the pituitary should really have been credited to the adrenal. On this account, in the review just offered, place has been given to the adrenal but not to the pituitary.

Since this is a book about rutin, and rutin is thought to affect both capillary fragility (see Chapter V) and permeability (see Chapter IV), it is proper to ask at this point whether either or both of these effects may be due to an action of rutin on the adrenal, on vitamin C, or on hyaluronidase.

Rutin might decrease fluid transfer through the capillary wall and mesenchymal ground substance: (1) by stimulating the adrenal medulla to produce more hormone or by, in some way, potentiating the amount of adrenal medullary hormone that is normally produced. This presumes, of course, that the adrenal medullary hormone does play a part in capillary permeability and fluid transfer through the pericapillary tissue. Such presumption is by no means proven. However, there is excellent evidence both in the work of Fuhrman *et al.*[229,230] and Wilson and DeEds[787,788,789] and by the French group[361] that rutin and other similarly acting substances do potentiate (i.e. increase the effectiveness and the duration of the effect) epinephrine. This may be accomplished by inhibiting the destruction of the epinephrine by oxidation. There is no evidence that rutin increases the production of epinephrine, and a point against this is failure of rutin administration to raise blood pressure; (2) by stimulating the adrenal cortex to produce more hormone of the Kendall Compound E type, or by potentiating, in some way, the action of the amount normally produced, although there is no evidence that it does either; (3) by depressing the production of the DOCA type hormone of the adrenal cortex, or by inhibiting its action once produced, but again there is no evidence that rutin does either; (4) by increasing the effectiveness of vitamin C. There is evidence that rutin does this as has been recorded in several papers (see Chapter V); (5) by

opposing the effect of hyaluronidase. While this problem has been studied, reports are contradictory (see Chapter IV).

Rutin may affect capillary fragility and permeability by a direct action of its own on the capillary wall and pericapillary tissue. This we think more likely than that its effectiveness lies only in motivating one of the above mentioned mechanisms.

Capillary Permeability and Movement of Lymph

As has been said in Chapter III, the walls of the capillaries consist of endothelial cells joined at their margins by what is usually referred to as the intercellular cement substance. In considering the transfer of fluid through the capillary wall, Chambers and Zweifach[92] concluded that the usual avenue of such transfer was through the intercellular substance and not through the cells. They came to this conclusion largely because transfer of fluid through a living cell body would result in increased work, and hence increased metabolism. When fluid passes through a cell body, as in the case of secreting epithelial cells, such increased metabolism does occur and can be measured. Because no such increase in metabolism can be demonstrated for endothelial cells when increased passage of fluid through the capillary walls occurs, it can be concluded that the actual transfer takes place through the intercellular substance, which functions as an inert membrane.

Cohn and his associates,[112,113] however, have shown that this intercellular membrane, permeable to electrolytes in solution, also permits the passage of certain large protein molecules through preformed pores. While these protein molecules differ in size, their diameter is fairly constant and their principal variation is in length. Since the direction of the long axis may vary at the time when the tip enters the pore, it is obvious that the shorter the length the more readily the molecule will pass through the pore. At any given time, however, a certain number of pores will be more or less congested by protein molecules passing through them. Any unfilled pores will be free to assist in the transfer of non-protein fluid through the capillary wall. Therefore, any condition that might be associated with a diminished number of protein molecules in the blood (especially of the long molecules which take longer to pass through the pores) could mechanically lead to an increased loss of fluid through the capillary wall, i.e. an increase in capillary permeability. This would be true even if the capillary wall itself were structurally normal. Moreover, a decrease in the number of protein molecules in the blood would be associated with a decrease in the colloidal osmotic pressure of the plasma. Colloidal osmotic pressure is a factor in the

The effect of flavonoid therapy in this study upon mortality of patients with capillary fault is illustrated by bar graphs in Figure 44. The prophylactic effect of these compounds upon mortality, that is, the comparative percentage of expected deaths in untreated capillary fault cases, as compared to both rutin and quercetin treated subjects, cannot be denied. The difference between the values obtained for quercetin and rutin is probably not significant because of the less extensive study with quercetin and due to the fact that the maximum dosage level for this flavonoid has not, as yet, been fully utilized.

Conclusions (Quercetin Therapy)

From these studies it would appear that quercetin is superior to rutin in the treatment of initial spontaneous capillary fault associated with hypertension. It is effective at lower dosage and appears to correct capillary fault in all cases which do not respond to rutin therapy. Also, quercetin corrects capillary fault and may be given to patients whose capillary dysfunction was successfully treated with rutin without risk of a relapse.

Bibliography

1. Abt, A. F., Farmer, C. J., and Epstein, I. M., *J. Pediat., 8,* 1 (1936).
2. Akamatsu, K., *Jap. J. Med. Sci.,* Sect. 4, *4* (1929). *Proc. Jap. Pharm. Soc. Ann. Meet.,* July 1929, p. 48.
3. Akamatsu, K., *Jap. J. Med. Sci.,* Sect. 4, *5* (1931). *Proc. Jap. Pharm. Soc. Ann. Meet.,* April 1930, p. 80.
4. Aleixo, J., *Brasil-Médico.* Rio de Janeiro, *62,* 361 (1948).
5. Alexander, B., and de Vries, A., *J. Clin. Invest., 28,* 24 (1949).
6. Alexander, B., de Vries, A., Goldstein, R., and Landwehr, G., *Science, 109,* (1949).
7. Alford, W. C., Shapiro, L., and White, C. E., *Anal. Chem., 23,* 1149 (1951).
8. Allen, E. V., Hines, E. A., Jr., Kvale, W. F., and Barker, N. W., *Ann. Inter Med., 27,* 371 (1947).
9. Allen, J. G., Bogardus, G., Jacobson, L. O., and Spurr, C. L., *Ann. Internal M 27,* 382 (1947).
10. Allen, J. G., Grossman, B. J., Elghammer, R. M., Moulder, P. V., McKeen, C. Sanderson, M., Egner, W., and Crosbie, J. M., *Surg. Gynec. and Obst., 89,* (1949).
11. Allen, J. G., Moulder, P. V., Elghammer, R. M., Grossman, B. J., McKeen, C. Sanderson, M., Egner, W., and Crosbie, J. M., *J. Lab. & Clin. Med., 34,* (1949).
12. Allen, J. G., Moulder, P. V., McKeen, C. L., Egner, W., Elghammer, R. M., Grossman, B. J., *Proc. Soc. Exptl. Biol. Med., 70,* 644 (1949).
13. Allen, J. G., Sanderson, M., Milham, M., Kirschon, A., and Jacobson, L. O., *Exp. Med., 87,* 71 (1948).
14. Ambrose, A. M., and DeEds, F., *J. Pharmacol. Exp. Therap.,* (a) *90,* 359 (194 (b) *97,* 115 (1949).
15. Ambrose, A. M., and DeEds, F., *J. Nutrition, 38,* 305 (1949).
16. Ambrose, A. M., Robbins, D. J., and DeEds, F., *Fed. Proc., 9,* 254 (1950).
17. Ambrose, A. M., Robbins, D. J., and DeEds, F., *J. Am. Pharm. Assoc., Sci. E 41,* 119 (1952).
18. Andersen, A. A., and Berry, J. A., *Science, 106,* 644 (1947).
19. Anderson, G., Unpublished data quoted by Göthlin, *Lancet,* 1937, II, 703.
20. Ané, J. N., and Burch, G. E., *Proc. Soc. Exptl. Biol. Med., 48,* 471 (1941).
21. Anon., *Science News Letter,* June 3, 1950, p. 344.
22. Anon., *Nutrition reviews 10,* 156 (1952).
23. Armentano, L., *Z. Ges. Exptl. Med. 102,* 219 (1938).
24. Armentano, L., Bentsáth, A., Béres, T., Rusznyák, I., and Szent-Györgyi, A., *Det Med. Wochschr. 62,* 1326 (1936).
25. Arons, I., Freeman, J., and Oppenheim, A., *Cancer Res. 11,* 234 (1951).

25a. Arriaga, C. J., *Arch. Soc. off. hispano-amer., Madr. 11*, 373 (1951).
26. Ashton, N., *Brit. J. Ophthalmology 34*, 38 (1950).
27. Attree, G. F., and Perkin, A. G., *J. Chem. Soc. 1927*, 234.
28. Auld, S. J. M., *Proc. Chem. Soc. 26*, 146 (1910).
29. Bacharach, A. L., and Coates, M. E., *Analyst 67*, 313 (1942).
30. Bacharach, A. L., Coates, M. E., and Middleton, T. R., *Biochem. J. 36*, 407 (1942).
31. Bacq, Z. M., Herve, A., Lecomte, J., and Fischer, P., *Science 111*, 356 (1950).
32. Badgett, C. O., Beinhart, E. G., Maher, Jeanne, and Connelly, J. A., *Archives of Biochemistry 24*, 245 (1949).
33. Balado, R. F., *Rev. fac. cienc. quím.* (Univ. nacl. La Plata) *23*, 155 (1948).
34. Balducci, Diego, *Boll. Soc. Ital. Biol. Sper. 24*, 243 (1948); *Chem. Abs. 42*, 7390.
35. Barker, M. H., *J. Am. Med. Assoc. 106*, 762 (1936).
36. Barnes, R. H., *Am. J. Med. Sci. 219*, 368 (1950).
37. Barron, E. S. G., Dickman, S., Muntz, J. A., and Singer, T. P., *J. Gen. Physiol. 32*, 537 (1949).
38. Bartlett, G. R., *J. Pharmacol. Exp. Therap. 93*, 329 (1948).
39. Bate-Smith, E. C., and Westall, R. G., *Biochim. Biophys. Acta 4*, 427 (1950).
40. Beardwood, J. T., Jr., Roberts, E., and Trueman, R., *Proc. Am. Diab. Assoc. 8*, 241 (1948).
40a. Beardwood, J. T., Jr., and Trueman, R. H., *J. Michigan State Med. Soc. 52*, 1074 (1953). *Am. J. Ophthalmol. 37*, 299 (1954).
41. Beaser, S. B., Rudy, A., and Seligman, A. M., *Arch. Internal Med. 73*, 18 (1944).
42. Beiler, J. M., Brendel, R., Graff, M., and Martin, G. J., *Arch. Biochem. 26*, 72 (1950).
43. Beiler, J. M., Brendel, R., and Martin, G. J., *Am. J. Pharm. 125*, 361 (1953).
44. Beiler, J. M., Graff, M., and Martin, G. J., *Am. J. Digest. Dis. 19*, 333 (1952).
45. Beiler, J. M., and Martin, G. J., *J. Biol. Chem. 171*, 507 (1947).
46. Beiler, J. M., and Martin, G. J., *J. Biol. Chem. 174*, 31 (1948).
47. Beiler, J. M., and Martin, G. J., *J. Biol. Chem. 192*, 831 (1951).
48. Belfus, F. H., and Madison, F. W., *J. Lab. Clin. Med. 33*, 1651 (1948).
49. Bell, G. H., Lazarus, S., and Munro, H. N., *Lancet* 1940, II, 155.
50. Bell, G. H., Munro, H. N., Lazarus, S., and Scarborough, H., *Lancet* 1942, II, 536.
51. Benditt, E. P., Schiller, S., Mathews, M. B., and Dorfman, A., *Proc. Soc. Exptl. Biol. Med. 77*, 643 (1951).
52. Bentsáth, A., and Das, N. B., *Z. f. Physiol. Chem. 247*, 258 (1937).
53. Bentsáth, A., Rusznyák, S., and Szent-Györgyi, A., *Nature 138*, 789 (1936); *139*, 326 (1937).
54. Bensáth, A., and Szent-Györgyi, A., *Nature 140*, 426 (1937).
55. Bentz, H., *Pharmazie 6*, 598 (1951); *Chem. Abs. 46*, 4127f.
56. Berka, I., and Nesvadba, O., *Časopis Českého Lékárnictva 63*, 33 (1950); *Chem. Abs. 46*, 4174a.
57. Bingham, J. B., Meyer, O. O., and Pohle, F. J., *Am. J. Med. Sci. 202*, 563 (1941).
58. Blaich, W., and Gerlach, U., *Zschr. Haut & Geschlkr. 13*, 145 (1952).
59. Blaich, W., and Tüshaus, B., *Ärztl. Wochschr. 5*, 696 (1950); *Chem. Abs. 45*, 2585g.
60. Blank, F., and Suter, R., *Exper. 4*, 72 (1948).
61. Blount, B. K., *J. Chem. Soc. London*, 1528 (1933).
62. Bognár, R., *Research* (London) *5*, 393 (1952); *Chem. Abs. 47*, 271h.
63. Bohr, D. F., McIvor, B. C., and Rinehart, J. F., *J. Pharmacol. Exp. Therap. 97*, 243 (1949).
63a. Booth, A. N., and DeEds, F., *J. Am. Pharm. Assoc., Sci. Ed. 40*, 384 (1951).
64. Bornträger, A., *Ann. Chem. Pharm. 53*, 385 (1845).
65. Boschi, E., and Gaspari, A., *Minerva med., Tor. 43*, 422 (1952).
66. Boullay, P. F. G., *Buchner's Rep. d. Pharm. 31*, 54 (cited by Schmidt, E., *Arch. Pharm. 246*, 214 (1908)).
67. Bourne, G. H., *Nature 152*, 659 (1943).
68. Bourne, G. H., *Brit. J. Nutrition 4*, xii (1950).
69. Boyer, P. D., Phillips, P. H., Pounden, W. D., Jensen, C. W., Rupel, I. W., and Nesbit, M. E., *J. Nutrition 23*, 525 (1942).
70. Bradfield, A. E., and Flood, A. E., *J. Chem. Soc.* 4740 (1952).
71. Brandl, J., and Schärtel, G., *Arch. Pharm. 250*, 414 (1912).
72. Brauns, D. H., *Arch. Pharm. 242*, 547, 556 (1904).
73. Brembach, H., *Münch. med. Wschr. 94*, 1475 (1952).
74. Bridel, M., and Beguin, D., *Bull. Soc. Chim. Biol. 8*, 901 (1926).
75. Brinkhous, K. M., *Proc. Soc. Exp. Biol. Med. 66*, 117 (1947).
76. Briskas, S., and Delbarre, F., *Presse Medicale 55*, 66 (1947).
77. Brown, E. E., *Am. Heart J. 34*, 241 (1947).
78. Brown, E. E., *J. Lab. & Clin. Med. 34*, 1714 (1949).
79. Brown, G. E., and O'Leary, P. A., *Arch. Internal Med. 36*, 73 (1925).
80. Brown, P. S., *Lancet 260*, 52 (1951).
81. Bruckner, V., and Szent-Györgyi, A., *Nature 138*, 1057 (1936).
82. Bryant, E. F., *J. Am. Pharm. Assoc., Sci. Ed. 39*, 480 (1950).
83. Burger, H., *Ztschr. Geburtsh. u. Gynäk. 135* (2), 182 (1951).
84. Burgess, J. F., *Lancet* 1948 (II) 215.
85. Burn, C. G., Orten, A. W., and Smith, A. H., *Yale J. Biol. Med. 13*, 817 (1941).
86. Bustinza, F., and Lopez, A. C., *an. Jardín Botanico de Madrid 7*, 549 (1946–1948).
87. Butler, S., Hall, F. R., and Sanford, H. N., *J. Lab. Clin. Med. 36*, 710 (1950).
87a. Cabo Torres, J., and Panadero Vidal, M., *Farmacognosia* (Madrid) *11*, 305 (1951).
88. Campbell, H., *Food Research 4*, 397 (1939).
89. Campbell, H. A., and Link, K. P., *J. Biol. Chem. 138*, 21 (1941).
90. Casteel, H. W., and Wender, S. H., *Anal. Chem. 25*, 508 (1953).
91. Chainey, J., U. S. Pat. No. 2,498,849 (1950).
92. Chambers, R., and Zweifach, B. W., *Physiol. Rev. 27*, 436 (1947).
93. Chang, M. C., and Pincus, G., *Science 117*, 274 (1953).
94. Chapman, W. H., and Cronkite, E. P., *Proc. Soc. Exptl. Biol. Med. 75*, 318 (1950).
95. Charaux, C., *Compt. Rend. Acad. Sci. Paris 178*, 1312 (1924).
96. Charaux, C., *Bull. Soc. Chim. Biol. 6*, 631 (1924).
97. Charaux, C., *Bull. Soc. Chim. Biol. 6*, 641 (1924).
98. Chrom, S. A., *Acta Radiol. 16*, 641 (1935).
99. Clark, W. G., *Proc. Soc. Exptl. Biol. Med. 69*, 97 (1948).
100. Clark, W. G., *Am. J. Physiol. 159*, 564 (1949). (Abstr.)
101. Clark, W. G., and Geissman, T. A., *Fed. Proc. 7*, 21 (1948).
102. Clark, W. G., and Geissman, T. A., *Nature 163*, 36 (1949).
103. Clark, W. G., and Geissman, T. A., *J. Pharmacol. Exp. Therap. 95*, 363 (1949).
104. Clark, W. G., and Jacobs, E., *Blood 5*, 320 (1950).
105. Clark, W. G., and MacKay, E. M., *Proc. Soc. Exptl. Biol. Med. 71*, 86 (1949).
106. Clark, W. G., and MacKay, E. M., *J. Allergy 21*, 133 (1950).
107. Clark, W. G., and MacKay, E. M., *J. Am. Med. Assoc. 143*, 1411 (1950).
108. Clark, W. G., Uncapher, R. P., and Jordan, M. L., *Science 108*, 629 (1948).
108a. Clarke, G., Jr., and Banerjee, S. C., *J. Chem. Soc. 97*, 1833 (1910).
109. Cohen, A., and Cohen, L., *Brit. J. Radiol. 25*, 601 (1952).
110. Cohen, L., *Brit. J. Radiol. 26*, 271 (1953).
111. Cohen, L., and Cohen, A., *Brit. J. Radiol. 26*, 551 (1953).

112. Cohn, E. J., *Ann. Internal Med. 26,* 341 (1947).
113. Cohn, E. J., Oncley, J. L., Strong, L. E., Hughes, W. L., Jr., and Armstrong, S. H., Jr., *J. Clin. Invest. 23,* 417 (1944).
114. Collins, R. A., Schreiber, M., and Elvehjem, C. A., *J. Nutrition 49,* 589 (1953).
115. Conley, C. L., Hartmann, R. C., and Lalley, J. S., *Proc. Soc. Exptl. Biol. Med. 69,* 284 (1948).
116. Conley, C. L., Hartmann, R. C., and Morse, W. I., II, *J. Clin. Invest. 28,* 340 (1949).
117. Conley, C. L., Rathbun, H. K., Morse, W. I., II, and Robinson, J. E., Jr., *Bull. Johns Hopkins Hosp. 83,* 288 (1948).
118. Cope, E. P., and Grover, R. W., *J. Invest. Dermatol. 10,* 39 (1948).
119. Cope, O., Brenizer, A. G., Jr., and Polderman, H., *Am. J. Physiol. 137,* 69 (1942).
120. Cope, O., and Moore, F. D., *J. Clin. Invest. 23,* 241 (1944).
121. Copley, A. L., *Science 107,* 201 (1948).
122. Copley, A. L., *Rutin. Processed Brochure* (36 p.). January 1949. (Available from J. Q. Griffith, Jr.)
123. Copley, A. L., and Houlihan, R. B., *Science 100,* 505 (1944).
124. Copley, A. L., and Lalich, J. J., *J. Clin. Invest. 21,* 145 (1942).
125. Cotereau, H., Gabe, M., Géro, E., and Parrot, J. L., *Nature 161,* 557 (1948).
126. Cotereau, H., Gabe, M., and Parrot, J. L., *Nature 158,* 343 (1946).
127. Cottafavi, N., Girardi, A., and Ponzoni, R., *Boll. Ist. sieroter., Milan 29,* 488 (1950).
128. Couch, J. F., U. S. Dept. Agr., Bur. Agr. Ind. Chem. AIC-192 (Eastern Regional Research Laboratory) December 1948 (Processed); Supplement 1, March 1951.
129. Couch, J. F., *Am. Chem. Soc. Jour. 70,* 256 (1948).
130. Couch, J. F., and Krewson, C. F., U. S. Dept. Agr., Bur. Agr. Ind. Chem. AIC-52 (Eastern Regional Research Laboratory) July 1944 (Processed).
131. Couch, J. F., and Krewson, C. F., U. S. Patent No. 2,453,305 (1948).
132. Couch, J. F., Krewson, C. F., and Naghski, J., U. S. Dept. Agr., Bur. Agr. Ind. Chem. AIC-160 (Eastern Regional Research Laboratory) July 1947 (Processed).
133. Couch, J. F., Krewson, C. F., and Naghski, J., U. S. Dept. Agr., Bur. Agr. Ind. Chem. AIC-202 (Eastern Regional Research Laboratory) July 1948 (Processed).
133a. Couch, J. F., Krewson, C. F., and Naghski, J., U. S. Dept. Agr., Bur. Agr. Ind. Chem. AIC-291 (Eastern Regional Research Laboratory) February 1951 (Processed).
134. Couch, J. F., Krewson, C. F., Naghski, J., and Copley, M. J., U. S. Dept. Agr., Bur. Agr. Ind. Chem. AIC-115 (Eastern Regional Research Laboratory) April 1946 (Processed).
135. Couch, J. F., Krewson, C. F., and Porter, W. L., U. S. Patent No. 2,500,930 (1950).
136. Couch, J. F., and Naghski, J., *J. Am. Chem. Soc. 67,* 1419 (1945).
137. Couch, J. F., Naghski, J., and Krewson, C. F., *Science 103,* 197 (1946).
138. Couch, J. F., Naghski, J., and Krewson, C. F., *J. Am. Chem. Soc. 74,* 424 (1952).
139. Couch, J. F., Naghski, J., and Porter, W. L., U. S. Patent No. 2,520,127 (1950).
140. Couch, J. F., Naghski, J., White, J. W., Taylor, J. W., Sando, W. J., and Street, O. E., U. S. Dept. Agr., Bur. Agr. Ind. Chem. AIC-222 (Eastern Regional Research Laboratory) February 1949 (Processed).
141. Craddock, C. G., and Lawrence, J. S., *J. Immunol. 60,* 241 (1948).
142. Crampton, E. W., and Lloyd, L. E., *Science 110,* 18 (1949).
143. Crampton, E. W., and Lloyd, L. E., *J. Nutrition 41,* 487 (1950).
144. Crandon, J. H., Lund, C. C., and Dill, D. B., *N. Eng. J. Med. 223,* 353 (1940).
145. Craver, B. N., *Am. J. Roentgenol. 59,* 404 (1948).
146. Criep, L. H., and Cohen, S. G., *Ann. Internal Med. 34,* 1219 (1951).
147. Criep, L. H., Levine, M. I., and Aaron, T. H., *Am. Rev. Tuberc. 59,* 701 (1949).
148. Crismon, J. M., Berez, R. R., Madden, J. D., and Fuhrman, F. A., *Am. J. Physiol. 164,* 391 (1951).
149. Cronkite, E. P., *Am. J. Path. 23,* 891 (1947).
150. Cronkite, E. P., Brecher, G., and Chapman, W. H., *Proc. Soc. Exptl. Biol. Med. 76,* 396 (1951).
151. Cronkite, E. P., and Chapman, W. H., *Proc. Soc. Exptl. Biol. Med. 74,* 337 (1950).
152. Cronkite, E. P., Chapman, W. H., and Brecher, G., *Proc. Soc. Exptl. Biol. Med. 76,* 456 (1951).
153. Cronkite, E. P., Chapman, W. H., and Chambers, F. W., *Proc. Soc. Exptl. Biol. Med. 76,* 282 (1951).
154. Cronkite, E. P., Eltzholtz, D. C., Sipe, C. R., Chapman, W. H., and Chambers, F. W., Jr., *Proc. Soc. Exptl. Biol. Med. 70,* 125 (1949).
155. Cronkite, E. P., Sipe, C. R., Eltzholtz, D. C., Chapman, W. H., and Chambers, F. W., Jr., *Proc. Soc. Exptl. Biol. Med. 73,* 184 (1950).
156. Cronkite, E. P., Tullis, J. L., Tessmer, C., and Ullrich, F. W., *Proc. Soc. Exptl. Biol. Med. 73,* 496 (1950).
157. Cruz-Coke, E., and Plaza de los Reyes, M., *Sociedad de la Biologia de Santiago de Chile, Boletin 4,* 19 (1947).
158. Cruz-Coke, E., and Plaza de los Reyes, M., *Sociedad de la Biologia de Santiago de Chile, Boletin 4,* 105 (1947).
159. Cruz-Coke, E., and Plaza de los Reyes, M., *Soc. de Chim. Biol. B. 29,* 573 (1947).
160. Cuseran, *Buchner's Rep. d. Pharm. 97,* 402 (as cited by Schmidt, E., *Arch. Pharm. 246,* 214 (1908)).
161. Cutter, I. S., and Johnson, C. A., *J. Am. Med. Assoc. 105,* 505 (1935).
162. Cutting, W. C., Dreisbach, R. H., Azima, M., Neff, B. J., Brown, B. J., and Wray, J., *Stanford Med. Bull. 9,* 236 (1951).
163. Cutting, W. C., Dreisbach, R. H., and Neff, B. J., *Stanford Med. Bull. 7,* 137 (1949).
164. Czimmer, A. G., *Arch. Exp. Path. Pharmakol. 183,* 587 (1936).
165. Dauer, M., and Coon, J. M., *Proc. Soc. Exptl. Biol. Med. 79,* 702 (1952).
166. Davis, E., *Am. J. Med. Sci. 212,* 192 (1946).
167. Davis, R. W., Dole, N., Izzo, M. J., and Young, L. E., *J. Lab. Clin. Med. 35,* 528 (1950).
168. Davis, W. B., Anal. Chem. *12,* 476 (1947).
169. Day, L. R., *Proc. Soc. Exptl. Biol. Med. 64,* 109 (1947).
170. Dechene, E. B., *J. Am. Pharm. Assoc., Sci. Ed. 40,* 93 (1951).
171. Dechene, E. B., *J. Am. Pharm. Assoc., Sci. Ed. 40,* 495 (1951)
172. DeCoursey, E., *Radiology 56,* 645 (1951).
173. DeEds, F., and Couch, J. F., *Food Res. 13,* 378 (1948).
174. Delâge, J. M., *Laval méd. 18,* 1372 (1953).
175. Diczfalusy, E., Ferno, O., Fex, H., Högberg, B., Linderot, T., and Rosenberg, T., *Acta Chem. Scandin. 7,* 913 (1953).
176. Dieckmann, W. J., Akbasli, Z., and Aragon, G. T., *Am. J. Obstet. Gynecol. 57,* 711 (1949).
177. Dixon, F. J., *Proc. Soc. Exptl. Biol. Med. 68,* 505 (1948).
178. Doenges, J. P., *Bull. School Med. Univ. Maryland 38,* 142 (1953).
179. Donegan, J. M., and Thomas, W. A., *Am. J. Ophthalmology 31,* 671 (1948).

180. Dontas, A. S., and Chenoweth, M. B., *Arch. intern. pharmacodynamie 91*, 202 (1952); *Chem. Abs. 47*, 2351d.
181. Dougherty, J., and Meneely, J. K., Jr., *J. Lab. Clin. Med. 38*, 709 (1951).
182. Doumer, E., Merlen, J. J., and Dubrulle, P., *Presse Med. 56*, 858 (1948).
183. Drinker, C. K., *Am. Heart J. 18*, 389 (1939).
184. Dumm, M. E., Ovando, P., Roth, P., and Ralli, E. P., *Proc. Soc. Exptl. Biol. Med. 71*, 368 (1949).
185. Dustin, P., and Gompel, C., *Compt. Rend. Soc. Biol. 143*, 874 (1949).
186. Dussy, J., *Compt. Rend. Acad. Sci. Paris 225*, 1368 (1947).
187. Eichna, L. W., *J. Clin. Invest. 21*, 731 (1942).
188. Eichna, L. W., *Am. Heart J. 25*, 812 (1943).
189. Eichna, L. W., and Bordley, J., III, *J. Clin. Invest. 21*, 711 (1942).
190. Eichna, L. W., and Wilkins, R. W., *J. Clin. Invest. 21*, 697 (1942).
191. Eidelmann, A., *Am. J. Physiol. 165*, 57 (1951).
192. El Ridi, M. S., and Aboul Wafa, M. H., *J. Egypt M. Ass. 36*, 1 (1953).
193. El Ridi, M. S., Strait, L. A., and Aboul Wafa, M. H., *Arch. Biochem. and Biophys. 39*, 317 (1952).
194. Ellinger, F., *Radiology 44*, 241 (1945).
195. Ellinger, F., *Science 104*, 502 (1946).
196. Ellinger, F., *Proc. Soc. Exptl. Biol Med. 64*, 31 (1947).
197. Ellinger, F., *Radiology 51*, 394 (1948).
198. Ellinger, F., *Proc. Soc. Exptl. Biol. Med. 74*, 616 (1950).
199. Ellinger, F., and Barnett, J. C., *Radiology 54*, 90 (1950).
200. Ellinger, F., Roswit, B., and Glasser, S. M., *Am. J. Roentgenol. & Rad. Therap. 61*, 387 (1949).
201. Elliott, R. H. E., *J. Am. Med. Assoc. 110*, 1177 (1938).
202. Elster, S. K., *Proc. Soc. Exptl. Biol. Med. 71*, 15 (1949).
203. Elster, S. K., Freeman, M. E., and Anderson, P. R., *J. Lab. Clin. Med. 34*, 834 (1949).
204. Elster, S. K., Freeman, M. E., and Dorfman, A., *Am. J. Physiol. 156*, 429 (1949).
204a. Elster, S. K., and Schack, J. A., *Am. J. Physiol. 161*, 283 (1950).
205. Enzinger, H., *Deut. Apoth.-Ztg. 92*, 288 (1952).
206. Ephrati, E., *Biochem. J. 42*, 383 (1948).
207. Eskew, R. K., Phillips, G. W. M., Griffin, E. L., Jr., and Edwards, P. W., U. S. Dept. Agr., Bur. Agr. & Ind. Chem. AIC-114 (Eastern Regional Research Laboratory) April 1946 (Processed).
208. Eskew, R. K., Phillips, G. W. M., Griffin, E. L., Jr., and Shaines, A., U. S. Dept. Agr., Bur. Agr. & Ind. Chem. (Eastern Regional Research Laboratory) AIC-114, Rev. 1, June 1948 (Processed).
209. Etcheverry, R., and France, O., *Sangre, Santiago 1*, 29 (1948); *Rev. Med. de Chile 76*, 331 (1948).
210. Eykman, J. F., *Rec. Trav. Chim. 5*, 127 (1886).
211. Fabianek, J., Neumann, J., and Lavollay, J., *Compt. rend. Acad. Sci. Paris 234*, 894 (1952).
212. Fahey, J. L., Olwin, J. H., and Ware, A. G., *Proc. Soc. Exptl. Biol. Med. 69*, 491 (1948).
212a. Faller, G., *Zahnarztl. Rdsch. 60*, 211 (1951).
213. Feinstein, R. N., Butler, C. L., and Hendley, D. D., *Science 111*, 149 (1950).
214. Fenske, C. S., Jr., and Couch, J. F., Unpublished data (Eastern Regional Research Laboratory).
215. Ferguson, J. H., and Lewis, J. H., *Proc. Soc. Exptl. Biol. Med. 67*, 228 (1948).
216. Field, J. B., and Rekers, P. E., *U. S. Atomic Energy Comm. MDDC* 1672 (Janu 1948).
217. Field, J. B., and Rekers, P. E., *Am. J. Med. Sci. 218*, 1 (1949).
218. Field, J. B., and Rekers, P. E., *J. Clin. Invest. 28*, 746 (1949).
219. Foerster, P., *Deut. Chem. Gesell. Ber. 15*, 214 (1882).
220. Fontaine, T. D., Ma, R., Poole, J. B., Porter, W. L., and Naghski, J., *Arch. chem. 15*, 89 (1947).
221. Foucar, H. O., *Canad. Med. Assoc. J. 59*, 21 (1948).
222. Foucar, H. O., *Canad. Med. Assoc. J. 60*, 402 (1949).
223. Fox, D. W., Savage, W. L., and Wender, S. H., *J. Am. Chem. Soc. 75*, 2504 (19
224. Fox, F. W., *Journal-Lancet, Minneapolis 63*, 349 (1943).
225. Frederic, J., *Arch. Biol. 60* (1), 79 (1949).
226. Frericks, C. T., Tillotson, I. G., and Hayman, J. M., Jr., *J. Lab. Clin. Med. 35*, (1950).
227. Friedemann, U., Traub, F. B., and Langstadt, D., *Proc. Soc. Exptl. Biol. Med.* 434 (1947).
228. Frommeyer, W. B., Jr., *J. Lab. Clin. Med. 34*, 1356 (1949).
229. Fuhrman, F. A., and Crismon, J. M., *J. Clin. Invest. 27*, 364 (1948).
230. Fuhrman, F. A., Crismon, J. M., Berez, R. R., and Madden, J. D., *Abstr Communs. 1st Intern. Congr. Biochem.* 1949, 52; *Chem. Abs. 45*, 6712g.
231. Fujise, S., and Tatsuta, H., *J. Chem. Soc. Japan, Pure Chem. Sect. 73*, 35 (19 *Chem. Abs. 47*, 3525h.
232. Fukuda, T., *Arch. Exptl. Path. Pharmakol. 164*, 685 (1932).
233. Fukuda, T., and Kono, M., *Jap. J. Med. Sci. Sect. 4*, 4 (1929). Abstracts pharmacological papers pub. in Japan, 1928, p. 27.
234. Furuya, K., Jap. Patent No. 1249 ('51), Mar. 6; *Chem. Abs. 47*, 276b.
235. Furuya, K., Jap. Patent No. 7246 ('51), Nov. 20; *Chem. Abs. 47*, 5079g.
236. Gabe, M., *Compt. Rend. Soc. Biol. 144*, 90 (1950).
237. Gabe, M., *Bull. histol. appl., Lyon 27*, 149 (1950).
238. Gabe, M., *Experientia 6*, 390 (1950).
239. Gabe, M., and Parrot, J. L., *J. de Physiol. 42*, 259 (1950).
240. Gabe, M., and Parrot, J. L., *Compt. Rend. Soc. Biol. 144*, 393 (1950).
241. Gabe, M., and Parrot, J. L., *Presse med. 59*, 1740 (1951).
242. Gabe, M., Parrot, J. L., and Cotereau, H., *Compt. Rend. Soc. Biol. 140*, 754 (19
243. Gabe, M., Parrot, J. L., and Cotereau, H., *Compt. Rend. Soc. Biol. 141*, 40 (19
243a. Gage, T. B., Douglass, C. D., and Wender, S. H., *Proc. Oklahoma Acad. Sci. 29*, (1948).
244. Gage, T. B., Douglass, C. D., and Wender, S. H., *Anal. Chem. 23*, 1582 (1951)
245. Gage, T. B., Morris, Q. L., Detty, W. E., and Wender, S. H., *Science 113*, (1951).
246. Gage, T. B., and Wender, S. H., *Fed. Proc. 8*, 293 (1949).
247. Gage, T. B., and Wender, S. H., *Anal. Chem. 22*, 708 (1950).
248. Gale, E. T., and Thewlis, M. W., *Geriatrics 8*, 80 (1953).
249. Galmiche, P., *Thesis. Univ. Paris* (1946), 144 p., Paris, Libraire le Francois.
250. Galmiche, P., *Sem. Hôp. Paris 22*, 1463 (1946).
251. Galston, A. W., *Science 111*, 619 (1950).
252. Gambacorta, O., *Med. Soc. N. Jersey J. 47*, 61 (Feb. 1950).
253. Garattini, S., and Genovese, E., *Atti. soc. lombarda sci. med. biol. 8*, 19 (1953).
254. Garino, M., *Z. Physiol. Chem. 88*, 1 (1913).
255. Geissman, T. A., and Hinreiner, E., *Botan. Rev. 18*, 77 (1952).
256. Géro, E., *Bul. Soc. Sci. Hyg. Aliment. 34*, 85 (1946).

257. Gershon-Cohen, J., Hermel, M. B., and Griffith, J. Q., Jr., *Science 114*, 157 (1951).
258. Glass, W. H., *Am. J. Med. Sci. 220*, 409 (1950).
259. Glazko, A. J., Adair F., Papageorge, E., and Lewis, G. T., *Science 105*, 48 (1947).
260. Goldstein, H. I., *Arch. Internal Med. 48*, 836 (1931).
261. Goldstein, D. H., Stolman, A., and Goldfarb, A. E., *Science 98*, 245 (1943).
262. Gollan, J., *Soc. de Chim. Biol. Bul. 11*, 1164 (1929).
263. Göthlin, G. F., *Skand. Arch. Physiol. 61*, 225 (1931).
264. Göthlin, G. F., *J. Lab. Clin. Med. 18*, 484 (1933).
265. Göthlin, G. F., *Acta Pediat. 20*, 71 (1937); also *Nord. Med. Tidskr. 14*, 1733 (1937); Abstract in *Lancet* 1937, II, 703.
266. Göthlin, G. F., Frisell, E., and Rundqvist, N., *Acta. Med. Scand. 92*, 1 (1937).
267. Graham, J. B., Graham, R. M., and Graffeo, A. J., *Endocrinology 46*, 431 (1950).
268. Grasset, J., Bret, J., and Cavagna, E., *Bull. Féd. soc. gn. obst. fr. 4*, 614 (1952).
269. Grasset, J., Bret, J., and Cavagna, E., *Sem. Hôp. Paris 29*, 542 (1953).
270. Greenblatt, R. B., *Obst. Gyn., N. Y. 2*, 530 (1953).
271. Greiling, G., *Med. Mschr. 5*, 775 (1951).
272. Greither, A., *Klin. Wochschr. 30*, 175 (1952).
273. Griffith, J. Q., Jr., *Proc. Inst. Med. Chicago 16*, 434 (Oct. 1947).
274. Griffith, J. Q., Jr., Scientific Exhibit, Am. Med. Assoc. 98th Annual Session, June 1949 (Information available from J. Q. Griffith, Jr.; or Eastern Regional Research Laboratory, Philadelphia 18, Pennsylvania).
275. Griffith, J. Q., Jr., *Mod. Med. 17* (11) 86, 94 (1949).
276. Griffith, J. Q., Jr., *J. Am. Pharm. Assoc., Sci. Ed. 42*, 68 (1953).
277. Griffith, J. Q., Jr., and Couch, J. F., Scientific Exhibit, Am. Med. Assoc. 97th Annual Session, June 1948 (Information available from J. Q. Griffith, Jr.; or Eastern Regional Research Laboratory, Philadelphia 18, Pennsylvania).
278. Griffith, J. Q., Jr., and Couch, J. F., *Blood 6*, 552 (1951).
279. Griffith, J. Q., Jr., Anthony, E., Pendergrass, E. P., and Perryman, R., *Proc. Soc. Exptl. Biol. Med. 64*, 331 (1947).
280. Griffith, J. Q., Jr., Anthony, E., Pendergrass, E. P., and Perryman, R., *Proc. Soc. Exptl. Biol. Med. 64*, 332 (1947).
281. Griffith, J. Q., Jr., Corbit, H. O., Rutherford, R. B., and Lindauer, M. A., *Am. Heart J. 21*, 77 (1941).
282. Griffith, J. Q., Jr , Couch, J. F., and Lindauer, M. A., *Proc. Soc. Exptl. Biol. Med. 55*, 228 (1944).
283. Griffith, J. Q., Jr., Fry, W. E., and Roberts, E., *Am. Heart J. 21*, 94 (1941).
284. Griffith, J. Q., Jr., Jeffers, W. A., and Fry, W. E., *Arch. Internal Med. 61*, 880 (1938).
285. Griffith, J. Q., Jr., and Lindauer, M. A., *Am. Heart J. 14*, 710 (1937).
286. Griffith, J. Q., Jr., and Lindauer, M. A., *Am. Heart J. 28*, 758 (1944).
287. Griffith, J. Q., Jr., and Lindauer, M. A., *Ohio State Med. J. 43*, 1136 (1947).
288. Griffith, J. Q., Jr., Lindauer, M. A., and Couch, J. F., Med. Soc. State of Penna. 93rd Ann. Sess., Oct. 1943, Program, p. 20.
289. Griffith, J. Q., Jr., Lindauer, M. A., Shanno, R. L., and Couch, J. F., Processed Brochure, 22 p., July 1946. (Available from J. Q. Griffith, Jr.; or Eastern Regional Research Laboratory, Philadelphia 18, Pennsylvania.)
290. Griffith, J. Q., Jr., Pendergrass, E. P., Perryman, C. R., and Hooker, J., *Proc. Soc. Exptl. Biol. Med. 61*, 70 (1946).
291. Griffith, J. Q., Jr., Roberts, E., Rutherford, R. B., and Corbit, H. O., *Am. Heart J. 21*, 62 (1941).
292. Gruber, C. M., Jr., and Tuft, L., *Ann. Allergy 11*, 288 (1953).
293. Gutiérrez-Moyano, L., *Galicia Clinica* (La Coruña, Spain) *21*, 117 (1949).
294. Hagedorn, P., and Neu, R., *Archiv. der Pharmazie 286*, 486 (1953).
295. Haizmann, R., and Blickle, B., *Deut. Med. J. 3*, 294 (1952).
296. Haley, T. J., and Bassin, M., *Proc. Soc. Exptl. Biol. Med. 81*, 298 (1952).
297. Haley, T. J., Clark, W. G., and Geissman, T. A., *Proc. Soc. Exptl. Biol. Med.* 202 (1947).
298. Haley, T. J., and Harris, D. H., *Science 111*, 88 (1950).
299. Haley, T. J., and Mann, S., *Proc. Soc. Exptl. Biol. Med. 81*, 665 (1952).
300. Haley, T. J., and Rhodes, B. M., *J. Am. Pharm. Assoc., Sci. Ed. 40*, 179 (19
301. Haley, T. J., Rhodes, B. M., Cassen, B., Curtis, L., and Kistler, K., *J. Am. Ph Assoc., Sci. Ed. 39*, 208 (1950).
302. Hallberg, O. E., *J. Am. Med. Assoc. 148*, 355 (1952).
303. Hanum, S., *Acta Ophthalmologica*, Supplementum XVI (1939), p. 110.
304. Hartmann, J., *Aerztl. Wschr. 8*, 407 (1953).
305. Hartwich, W., *Arquivos de Biologia 34*, 173 (1950).
306. Hasegawa, H., *J. Agr. Chem. Soc. Japan 7*, 1036 (1931).
307. Hayashi, K., and Ôuchi, K., *Proc. Japan Acad. 24*, 16 (1948); *Chem. Abs.* 4178a.
308. Heiman, M., *Arch. Ophthalmol. 28*, 493 (1942).
309. Hein, H., *Klin. Wochschr. 26*, 466 (1948).
310. Hein, H., *Arztliche Forschung 4*, I, 69 (1950).
311. Hein, H., and Laubmann, H., *Arztliche Forschung 4*, I, 666 (1950).
312. Hellerstein, H. K., Orbison, J. L., Rodbard, S., Wilburne, M., and Katz, L. *J. Lab. Clin. Med. 34*, 1608 (1949).
313. Hellerstein, H. K., Orbison, J. L., Rodbard, S., Wilburne, M., and Katz, L. *Am. Heart J. 42*, 271 (1951).
314. Hempelmann, L. H., Lisco, H., and Hoffman, J. G., *Ann. Internal Med. 36*, (1952).
315. Hench, P. S., Kendall, E. C., Slocumb, C. H., and Polley, H. F., *Proc. Staff M Mayo Clinic, 24* (8), 181 (1949); *Biol. Abstr. 24*, 12190.
316. Hennessy, T. G., and Huff, R. L., *Proc. Soc. Exptl. Biol. Med. 73*, 436 (1950).
317. Henshaw, P. S., *J. Nat. Cancer Inst. 4*, 503 (1944).
318. Hepding, L., *E. Merck's Jahresbericht 61/62*, 35 (1947/48).
319. Hepding, L., *Deut. Med. Wochschr. 74*, 1575 (1949).
320. Herrmann, H., Boss, M. B., and Friedenwald, J. S., *J. Biol. Chem. 164*, 773 (19
321. Herzig, J., *Monatsh. 5*, 72 (1884); *6*, 863 (1885); *9*, 537 (1888); *12*, 172 (189 *14*, 53 (1893); and *15*, 683 (1894).
322. Higby, R. H., *J. Am. Pharm. Assoc., Sci. Ed. 32*, 74 (1943).
323. Highstein, B., and Zeligman, I., *J. Am. Med. Assoc. 146*, 816 (1951).
324. Hiramatsu, N., *Jap. J. Dermat. Urol. 49*, (4), 286 (1941).
325. Hlasiewetz, H., *Ann. Chem. 96*, 123 (1855).
326. Hodes, P. J., and Griffith, J. Q., Jr., *Radiology 37*, 203 (1941).
327. Holden, W. D., Cole, J. W., Portman, A. F., and Storaasli, J. P., *Proc. Soc. Ex Biol. Med. 70*, 553 (1949).
328. Holland, A. H., Jr., Canniff, J. C., and Bruger, M., *J. Lab. Clin. Med. 32*, (1947).
329. Hollenhorst, R. W., and Wagener, H. P., *Am. J. Med. Sci. 217*, 223 (1949).
330. Holman, R. L., and Swanton, M. C., *Proc. Soc. Exptl. Biol. Med. 63*, 87 (1946
331. Hopps, H. C., and Lewis, J. H., *Am. J. Path. 23*, 829 (1947).
332. Hörhammer, L., and Hänsel, R., *Arch. Pharm. 284*, 276 (1951).
333. Hörhammer, L., and Hänsel, R., *Arch. Pharm. 285*, 438 (1952).

334. Hörhammer, L., and Hänsel, R., *Arch. Pharm. 286*, 425 (1953).
335. Hörhammer, L., and Hänsel, R., *Arch. Pharm. 286*, 447 (1953).
336. Hörhammer, L., Hänsel, R., and Frank, P., *Arch. Pharm. 286*, 481 (1953).
337. Hörhammer, L., Hänsel, R., and Strasser, F., *Arch. Pharm. 285*, 286 (1952).
338. Hörhammer, L., and Müller, K., *Arch. Pharm. 287*, 126 (1954).
339. Horne, G., and Scarborough, H., *Lancet* 1940, II, 66.
340. Howland, J. W., Furth, F., and Coulter, M., *Fed. Proc. 9*, 334 (1950).
341. Huff, R. L., Bethard, W. F., Garcia, J. F., Roberts, B. M., Jacobson, L. O., and Lawrence, J. H., *J. Lab. Clin. Med. 36*, 40 (1950).
342. Hughes, E. G., and Parkes, M. W., Jubilee Volume dedicated to Emil Christoph Barell. Frederick Reinhardt, Ltd. Co., Basle, Switzerland, p. 216 (1946).
343. Humble, J. G., *Blood 4*, 69 (1949).
344. Humphreys, F. R., *Australian J. Science 16*, 30 (1953).
345. Ibañez, J., *Bol. Soc. Biol.* (Santiago, Chile) *7*, 21 (1949).
346. Ibañez, J., Guiser, R., and Szabó, E., *Bol. Soc. Biol. 7*, 19 (1949).
347. Ibañez, J., and Orellana, G., *Farm. Nueva* (Madrid) *15*, 283 (1950).
348. Ice, C. H., and Wender, S. H., *Anal. Chem. 24*, 1616 (1952).
349. Ice, C. H., and Wender, S. H., *J. Am. Chem. Soc. 75*, 50 (1953).
350. Imai, K., and Furuya, K., *J. Pharm. Soc. Japan 71*, 266 (1951).
351. Isaacs, R., *J. Am. Med. Assoc. 121*, 1306 (1943).
352. Itallie, L., von, *Pharm. Weekbld. 55*, 709 (1918); *Chem. Abs. 12*, 1891.
353. Ivančević, I., and Knežević, M., *Arch. internat. pharm. dyn. Ghent 86*, 414 (1951).
354. Jacobson, L. O., Marks, E. K., Gaston, E. O., Robson, M., and Zirkle, R. E., *Proc. Soc. Exptl. Biol. Med. 70*, 740 (1949).
355. Jacobson, L. O., Marks, E. K., Robson, M. J., Gaston, E. O., and Zirkle, R. E., *J. Lab. Clin. Med. 34*, 1538 (1949).
356. Jacobson, L. O., Simmons, E. L., Bethard, W. F., Marks, E. K., and Robson, M. J., *Proc. Soc. Exptl. Biol. Med. 73*, 455 (1950).
357. Jacobson, L. O., Simmons, E. L., and Block, M. H., *J. Lab. Clin. Med. 34*, 1640 (1949).
358. Jacobson, L. O., Simmons, E. L., Marks, E. K., and Eldredge, J. H., *Science 113*, 510 (1951).
359. Jacobson, L. O., Simmons, E. L., Marks, E. K., Robson, M. J., Bethard, W. F., and Gaston, E. O., *J. Lab. Clin. Med. 35*, 746 (1950).
360. Jacox, R. F., and Bays, R. P., *Proc. Soc. Exptl. Biol. Med. 70*, 587 (1949).
361. Javillier, M., and Lavollay, J., *Helv. Chim. Acta 29*, 1283 (1946).
362. Jeney, A. v., and Czimmer, A. G., *Arch. Exptl. Path. Pharmakol. 183*, 571 (1936).
363. Jeney, A. v., and Czimmer, A. G., *Arch. Exptl. Path. Pharmakol. 190*, 648 (1938).
364. Jeney, A. v., Méhes, G., Czimmer, A. G., and Sokoray, L., *Arch. Exptl. Path. Pharmakol. 187*, 553 (1937).
365. Jeney, E., *Wien. Z. Inn. Med. 28*, 165 (1947).
366. Jermstad, A., and Jensen, K. B., *Bull. Soc. Chim. Biol. 33*, 258 (1951).
367. Johne, H. O., *Arch. Derm. Syph., Berl. 194*, 287 (1952).
368. Jonsson, A. G., Obel, A. L., and Sjöberg, K., *Z. Vitaminforsch. 12*, 300 (1942).
369. Jorpes, J. E., *Ann. Internal Med. 27*, 361 (1947).
370. Jubelirer, R. A., and Glueck, H. I., *J. Lab. Clin. Med. 34*, 448 (1949).
371. Kakemi, K., Uno, T., and Iwama, M., *J. Pharm. Soc. Japan 73*, 101 (1953).
372. Kariyone, T., Hashimoto, Y., Mori, I., and Kimura, M., *J. Pharm. Soc. Japan 73*, 1095 (1953).
373. Karrer, W., *Helv. Chim. Acta 32*, 714 (1949).
374. Katô, H., *Folia Pharmacol. Japon. 47*, No. 3/4, 93 (1951). Breviaria, p. 7.
375. Kato, Y., *Igaku to Seibutsugaku* (Med. & Biol.) *16*, 280 (1950); *Chem. Abs.* 1249g.
376. Kay, J. H., Hutton, S. B., Balla, G., and Ochsner, A., Scientific Exhibit, Am. M Assoc. 99th Ann. Sess. June 1950, San Francisco. *J. Am. Med. Assoc. 143*, 2 (1950).
377. Keenan, G. L., *J. Am. Pharm. Assoc., Sci. Ed. 37*, 479 (1948).
378. Kelly, L. S., and Jones, H. B., *Proc. Soc. Exptl. Biol. Med. 74*, 493 (1950).
379. Keuning, F. J., and van der Slikke, L. B., *J. Lab. Clin. Med. 36*, 167 (1950).
380. Khatwa, H. A., and Kinawi, M., *J. Roy. Egypt. Med. Assoc. 33*, 387 (1950); *Ch Abs. 44*, 9527.
381. Kimeldorf, D. J., Jones, D. C., and Fishler, M. C., *Science 112*, 175 (1950).
382. King, B. C., and Schwarting, A. E., *J. Am. Pharm. Assoc., Sci. Ed. 38*, 531 (194
383. Kirtley, W. R., and Peck, F. B., *Am. J. Med. Sci. 216*, 64 (1948).
384. Klemperer, P., *Ann. Internal Med. 28*, 1 (1948).
385. Knauer, W. J., *Florida Med. Assoc. J. 37*, 88 (1950).
386. Knüchel, F., and Kienle, F., *Ärztliche Forschung 4*, I, 81 (1950).
387. Kobayashi, K., *J. Pharm. Soc. Japan 71*, 1493 (1951).
388. Kohn, H. I., *Am. J. Physiol. 165*, 43 (1951).
389. Kohn, H. I., Robinett, P. W., and Cupp, M. N., *U. S. Atomic Energy Com AECD* 2176 (Nov. 1948).
389a. Koike, H., *Folia Pharmacol. Japon. 12*, 89 (1931). Breviaria, p. 7.
390. Kono, M., *Jap. J. Med. Sci. Sect. 4*, 4 (1929). Abstracts of pharmacologi papers pub. in 1928, p. 26.
391. Kono, M., *Jap. J. Med. Sci. Sect. 4*, 4 (1929). Abstracts of pharmacologi papers pub. in 1928, p. 27.
392. Koones, H. F., U. S. Patent No. 2,450,555 (1948).
393. Krasno, L. R., and Ivy, A. C., *Circulation 1*, 1267 (1950).
394. Krasno, L. R., and Ivy, A. C., Personal communication to the authors.
395. Krewson, C. F., U. S. Patent No. 2,543,783 (1951).
396. Krewson, C. F., U. S. Patent No. 2,637,725 (1953).
397. Krewson, C. F., and Couch, J. F., *Am. Chem. Soc. J. 70*, 257 (1948).
398. Krewson, C. F., and Couch, J. F., *J. Am. Pharm. Assoc., Sci. Ed. 39*, 163 (1950
399. Krewson, C. F., and Couch, J. F., *J. Am. Pharm. Assoc., Sci. Ed. 41*, 83 (1952
400. Krewson, C. F., Fenske, C. S., Jr., Couch, J. F., and Naghski, J., *Am. J. Phar 125*, 117 (1953).
401. Krewson, C. F., and Naghski, J., *J. Am. Pharm. Assoc., Sci. Ed. 41*, 582 (1952
402. Krewson, C. F., and Naghski, J., *Am. J. Pharm. 125*, 190 (1953).
403. Krewson, C. F., Naghski, J., and Porter, W. L., U. S. Patent No. 2,534,275 (1950
404. Krieger, H., Holden, W. D., Hubay, C. A., Scott, M. W., Storaasli, J. P., a Friedell, H. L., *Proc. Soc. Exptl. Biol. Med. 73*, 124 (1950).
405. Küchle, H. J., and Wegener, H., *Zschr. ges. exp. Med. 118*, 136 (1951).
406. Küchmeister, H., *E. Merck's Jahresbericht 61/62*, 26 (1947/48).
407. Küchmeister, H., *Klin. Wochschr. 27*, 297 (1949).
408. Kugelmass, I. N., *J. Am. Med. Assoc. 115*, 519 (1940).
409. Kugelmass, I. N., *Arch. Otolaryngology 46*, 684 (1947).
410. Kuhn, R., and Löw, I., *Chem. Ber. 81*, 363 (1948).
411. Kuhn, R., and Löw, I., *Chem. Ber. 82*, 474 (1949).
412. Kuhn, R., Moewus, F., and Löw, I., *Ber. Deut. Chem. Ges. 77B*, 219 (1944).
413. Kühnau, J., *E. Merck's Jahresbericht 61/62*, 8 (1947/48).
414. Kühnau, J., *Klin. Wochschr. 27*, 294 (1949).
415. Kunkler, A. W., and Shumacker, H. B., Jr., *Angiology 5*, 11 (1954).

415a. Kurth, E. F., *Tappi 36*, 119A (1953).
415b. Kurth, E. F., and Chan, F. L., *J. Am. Leather Chemists' Assoc. 48*, 20 (1953).
416. Kuschinsky, G., *Klin. Wochschr. 27*, 317 (1949).
417. Kuschinsky, G., DuPont, W., and Hennes, R., *Arch. Exptl. Path. Pharmakol. 207*, 138 (1949).
418. Kuschinsky, G., and Ludewig, H., *Klin. Wochschr. 28*, 138 (1950).
419. Kushlan, S. D., *Gastroenterology 7*, 199 (1946).
420. Kushlan, S. D., *Angiology 4*, 346 (1953).
421. Kutscher, A. H., *Am. J. Obst. 61*, 1318 (1951).
422. Landis, E. M., *Am. J. Physiol. 82*, 217 (1927).
423. Landis, E. M., *Heart, 15*, 209 (1930).
424. Landis, E. M., and Gibbon, J. H., Jr., *J. Clin. Invest. 12*, 105 (1933).
425. Lange, K., and Krewer, S. E., *J. Lab. Clin. Med. 28*, 1746 (1943).
426. Lavollay, J., *Compt. Rend. Soc. Biol. 135*, 1193 (1941).
427. Lavollay, J., and Neumann, J., *Compt. Rend. Acad. Sci. Paris 212*, 251 (1941).
428. Lavollay, J., and Neumann, J., *Exposés annuels biochim. méd. 10*, 59 (1949).
429. Lavollay, J., Parrot, J. L., and Sevestre, J., *Compt. Rend. Acad. Sci. Paris 217*, 540 (1943).
430. Lawrence, G. H., *Endocrinology 45*, 383 (1949).
431. Lawrence, J. H., and Tennant, R., *J. Exptl. Med. 66*, 667 (1937).
432. Lawson, H. C., Carne, H. O., and Thienes, C. H., *Proc. Soc. Exptl. Biol. Med. 54*, 327 (1943).
433. Lazarus, S., Munro, H. N., and Bell, G. H., *Clin. Science 7*, 175 (1948).
434. LeBlond, C. P., and Segal, G., *Am. J. Roentgenol. 47*, 302 (1942).
435. Leclercq, R., *Gaz. med. France 57*, 1267 (1950).
436. Lecoq, R., *Compt. Rend. Soc. Biol. 142*, 118 (1948).
437. Lecoq, R., *Compt. Rend. Acad. Sci. Paris 235*, 585 (1952).
438. Lecoq, R., Chauchard, P., and Mazoué, H., *Compt. Rend. Soc. Biol. 141*, 52 (1947).
439. Lecoq, R., Chauchard, P., and Mazoué, H., *Thérapie 3*, 148 (1948); *Chem. Abs. 47*, 3943g.
440. Lee, R. E., and Lee, N. Z., *Am. J. Physiol. 149*, 465 (1947).
441. Levcowich, T., and Batchelder, E. L., *J. Nutrition 23*, 399 (1942).
442. Levin, O. L., Silvers, S. H., and Berkowitz, S. S., *Arch. Dermat. Syphil. 36*, 1176 (1937).
443. Levitan, B. A., *Proc. Soc. Exptl. Biol. Med. 68*, 566 (1948).
444. Levitan, B. A., *Proc. Soc. Exptl. Biol. Med. 68*, 569 (1948).
445. Levitan, B. A., *Am. J. Physiol. 157*, 422 (1949).
446. Levitan, B. A., *N. Eng. J. Med. 241*, 780 (1949).
447. Levitan, B. A., *Am. J. Med. Sci. 221*, 185 (1951).
448. Levitt, L. M., Cholst, M. R., King, R. S., and Handelsman, M. B., *Am. J. Med. Sci. 215*, 130 (1948).
449. Lewis, R. B., and Moen, P. W., *Exp. Med. and Surg.* (Basel) *11*, 9 (1953).
450. Liebermann, C., and Hamburger, S., *Ber. 12*, 1178 (1879).
451. Liebow, A. A., and Warren, S., *Am. J. Path. 23*, 888 (1947).
452. Liebow, A. A., Warren, S., and DeCoursey, E., *Am. J. Path. 25*, 853 (1949).
453. Limperos, G., and Mosher, W. A., *Science 112*, 86 (1950).
453a. Link, K. P., *Federation Proc. 4*, 176 (1945).
454. Lloyd, J. U., *Am. Jour. Pharm. 93*, 40 (1921).
455. Lloyd, J. U., and Lloyd, C. G., *Pharm. Rundsch. New York 4*, 224 (1886).
456. Loomis, T. A., *J. Lab. Clin. Med. 34*, 631 (1949).
457. Lorenz, A. J., and Arnold, L. J., *Food Res. 6*, 151 (1941).
458. Loughlin, W. C., *N. Y. State J. Med. 49*, 1823 (1949).
458a. Ma, R., and Fontaine, T. D., *Arch. Biochem. 16*, 399 (1948).
459. McGregor, W. G., and McKillican, M. E., *Sci. Agric. 32*, 48 (1952).
460. MacLean, A. L., and Brambel, C. E., *Am. J. Ophthalmology 30*, 1093 (1947).
461. McManus, J. F., and Landrigan, F. L., *Proc. Am. Fed. Clin. Res.* (1946) *3*, (1947).
462. McMaster, P. D., *J. Exptl. Med. 65*, 347 (1937).
463. McMaster, P. D., *J. Exptl. Med. 65*, 373 (1937).
464. McMaster, P. D., and Parsons, R. J., *J. Exptl. Med. 68*, 377 (1938).
465. MacWillie, J., *Biol. Human Affairs 10*, 33 (1944); *Chem. Abs. 41*, 6316d.
466. Madison, F. W., and Belfus, F. H., *Rutin. Processed Brochure* (36 p.) (Jan. 194 (Available from J. Q. Griffith, Jr.)
466a. Madison, F. W., and Pohle, H. W., *J. Lab. Clin. Med. 32*, 340 (1947).
467. Mager, A., *Z. Physiol. Chem. 274*, 109 (1942).
468. Maiden, J. H., *J. Proc. Roy. Soc. N.S.W. 21*, 250 (1887) (cited by Rodwell[616]).
469. Malhuret, R., *Bull. Soc. fr. derm. syph., Par. 57*, 490 (1950).
470. Malkiel, S., and Werle, M. D., *Science 114*, 98 (1951).
471. Mandelin, K. von, *Pharm. Ztschr. f. Russl.* 1883, 329 (cited by *Ber. 16*, 16 (1883)).
472. Maratka, Z., and Ivy, A. C., *Gastroenterology 11*, 357 (1948).
473. Markoff, N., *Schweiz. Med. Wochschr. 78*, 984 (1948).
474. Marshall, W., *Am. J. Surg. 80*, 52 (1950).
475. Martin, G. J., *Science 117*, 363 (1953).
476. Martin, G. J., and Beiler, J. M., *Science 115*, 402 (1952).
477. Martin, G. J., Carley, J., Jr., and Moss, J. N., *Exptl. Med. and Surg. 7*, 391 (1949
478. Martin, G. J., Graff, M., Brendel, R., and Beiler, J. M., *Arch. Biochem. 21*, 17 (1949).
479. Martin, G. J., Moss, J., and Beiler, J. M., *Proc. Soc. Exptl. Biol. and Med. 84*, 30 (1953).
480. Martin, G. J., and Swayne, V., *Science 109*, 201 (1949).
480a. Martini, G. A., and Engelkamp, H., *Deut. Med. Wschr. 77*, 833 (1952).
481. Martius, T., *Arch. Pharm. 160*, 231 (1862).
482. Marx, R., and Bayerle, H., *Ärztliche Forschung 4*, I, 250 (1950).
483. Masereé, M., and Paris, R., *Bull. Sci. Pharmacol. 43*, 279 (1936).
484. Masquelier, J., *Bull. Soc. Chim. Biol. 33*, 302 (1951).
485. Masquelier, J., *Bull. Soc. Chim. Biol. 33*, 304 (1951).
486. Masquelier, J., and Tayeau, F., *Bull. trav. soc. pharm. Bordeaux 88*, 168 (1950).
487. Matis, P., *Deut. Med. Wochschr. 74*, 1576 (1949).
488. Matis, P., Funfack, H., and Rockstroh, C., *Schweiz. Med. Wochschr. 80*, 70 (1950).
489. Matis, P., and Wundt, W., *Arch. klin. chir. 268*, 436 (1951).
490. Maxwell, R. D., and McDonnel, G. M., Unpublished data (Presented before the Fed. Am. Soc. Exptl. Biol., Atlantic City (1950)).
491. Mayer, J., and Krehl, W. A., *Arch. Biochem. 16*, 313 (1948).
492. Mayer, J., and Krehl, W. A., *J. Nutrition 35*, 523 (1948).
493. Menville, L. J., and Ané, J. N., *Am. J. Roentgenol. 31*, 166 (1934).
494. Mester, L., *Magyar Chem. Folyóirat 50*, 125 (1944).
495. Meyer, K., *Physiol. Rev. 27*, 335 (1947).
496. Meyer, K., and Ragan, C., *Mod. Concepts Cardiovas. Dis. 17*, No. 2, 2 p., Feb. (1948).
497. Miller, C. P., Hammond, C. W., and Tompkins, M., *Science 111*, 540 (1950).

498. Miller, C. P., Hammond, C. W., and Tompkins, M., *Science 111*, 719 (1950).
499. Millman, N., and Rosen, F., *Science 118*, 212 (1953).
500. Moewus, F., and Deulofeu, V., *Nature 173*, 218 (1954).
501. Moll, T., *Klin. Wochschr. 16*, 1653 (1937).
502. Moloney, W. C., *Am. J. Med. Sci. 205*, 229 (1943).
503. Monto, R. W., Bales, D. W., and Brennan, M. J., *J. Michigan M. Soc. 52*, 62 (1953).
504. Morris, Q. L., Gage, T. B., and Wender, S. H., *J. Am. Chem. Soc. 73*, 3340 (1951).
504a. Morrow, C. A., and Sandstrom, W. M., "Biochemical Laboratory Methods for Students of the Biological Sciences," John Wiley & Sons, Inc., New York, 1935, p. 295.
505. Moss, J. N., Beiler, J. M., and Martin, G. J., *Science 112*, 16 (1950).
506. Mouriquand, G., and Edel, V., *Intern. Z. Vitaminforsch. 22*, 129 (1950).
507. Mouriquand, G., and Edel, V., *Intern. Z. Vitaminforsch. 22*, 133 (1950).
508. Muller, H. J., *Am. Scientist 38*, 399 (1950).
509. Munro, H. N., Lazarus, S., and Bell, G. H., *Nutrition Abs. and Revs. 17*, 291 (1947).
510. Murray, C. W., Booth, A. N., DeEds, F., and Wilson, R. H., *J. Am. Chem. Soc. 75*, 3289 (1953), *J. Am. Pharm. Assoc., Sci. Ed. 43*, 361 (1954).
511. Muschaweck, R., *Arch. Exptl. Path. Pharmakol. 209*, 279 (1950).
512. Mylius, E., *Arch. Pharm. 201*, 97 (1872).
513. Naghski, J., Brice, B. A., and Krewson, C. F., *Am. J. Pharm. 124*, 297 (1952).
514. Naghski, J., Copley, M. J., and Couch, J. F., *Science 105*, 125 (1947).
515. Naghski, J., Copley, M. J., and Couch, J. F., *J. Bact. 54*, 34 (1947).
516. Naghski, J., Fenske, C. S., Jr., and Couch, J. F., *J. Am. Pharm. Assoc., Sci. Ed. 40*, 613 (1951).
517. Naghski, J., Fenske, C. S., Jr., Krewson, C. F., and Couch, J. F., U. S. Dept. Agr., Bur. Agr. Ind. Chem. AIC-236 (Eastern Regional Research Laboratory) August 1949 (Processed).
518. Naghski, J., and Krewson, C. F., *J. Am. Pharm. Assoc., Sci. Ed. 42*, 66 (1953).
519. Naghski, J., Krewson, C. F., Fenske, C. S., Jr., and Couch, J. F., (Unpublished data), Eastern Utilization Research Branch, Agricultural Research Service, U.S.D.A.
520. Naghski, J., Krewson, C. F., Porter, W. L., and Couch, J. F., *J. Am. Pharm. Assoc., Sci. Ed. 39*, 696 (1950).
521. Naghski, J., Mellon, E. F., Korn, A. H., and Ogg, C. L., *J. Am. Pharm. Assoc., Sci. Ed. 41*, 599 (1952).
522. Naghski, J., Porter, W. L., and Couch, J. F., *J. Am. Chem. Soc. 69*, 572 (1947).
522a. Nakabayashi, T., *J. Agr. Chem. Soc. Japan 27*, 274 (1953).
523. Nakamura, Y., Jap. Patent No. 7146 ('51) Nov. 13; *Chem. Abs. 47*, 50791.
524. Nakaoki, T., *J. Pharm. Soc. Japan 52*, 195 Dec. (1932).
525. Nakaoki, T., *J. Pharm. Soc. Japan 69*, 321 (1949); *Chem. Abs. 44*, 1977.
526. Neuberg, C., and Kobel, M., *Naturwiss. 23*, 800 (1935).
527. Neuberg, C., and Kobel, M., *Enzymologia 1*, 177 (1936).
528. Neumann, J., Fabianek, J., and Lavollay, J., *Compt. Rend. Acad. Sci. Paris 234*, 1407 (1952).
529. Nichele, G., and Carlino, L., *Arch. ital. pediat. 14*, 351 (1951).
530. Nichol, E. S., *Ann. West. Med. Surg. 4*, 71 (1950).
531. Niedermeier, S., *Klin. Mbl. Augenh. 121*, 559 (1952).
532. Niô, S., and Wada, E., *J. Agr. Chem. Soc. Japan 24*, 485 (1951).
532a. Nystrom, C. W., Williams, B. L., and Wender, S. H., *J. Am. Chem. Soc. 76*, 1950 (1954).
533. Oiseth, D., and Nordal, A., *Pharm. Acta Helv. 27*, 361 (1953).
534. O'Neill, J. F., *Ann. Surg. 126*, 270 (1947).
535. Orbison, J. L., and Peters, E., *Proc. Soc. Exptl. Biol. Med. 83*, 173 (1953).
536. Oshima, Y., and Nakabayashi, T., *J. Agr. Chem. Soc. Japan 25*, 212 (1951–5
537. Oshima, Y., Nakabayashi, T., and Imagawa, H., *J. Agr. Chem. Soc. Japan 25*, (1951–52).
538. Owen, C. A., and Bollman, J. L., *Proc. Soc. Exptl. Biol. Med. 67*, 231 (1948).
539. Owen, C. A., Jr., and Bollman, J. L., *Proc. Soc. Exptl. Biol. Med. 67*, 367 (19
540. Owren, P. A., *Acta Med. Scand. Suppl. 194*, 327 pp. (1947).
541. Ozawa, H., *Folia Pharmacol. Japan. 47*, 85 (1951); *Chem. Abs. 46*, 8264h.
542. Ozawa, H., and Okuda, T., Jap. Patent No. 1677 ('50) June 7; *Chem. Abs.* 8334i.
543. Ozawa, H., and Okuda, T., *Yakugaku* (Science of Drugs) *4*, 264 (1950).
544. Ozawa, H., and Okuda, T., Jap. Patent No. 1221 ('51) March 6; *Chem. Abs.* 276b.
545. Ozawa, H., Okuda, T., and Matsumoto, S., *J. Pharm. Soc. Japan 71*, 1173 (195
546. Palmer, L. J., Flaherty, N. F., Crampton, J. H., and Johnson, R. H., *North Medicine* (Seattle) *50*, 669 (1951).
547. Palos, L. A., *Proc. Soc. Exptl. Biol. Med. 71*, 471 (1949).
548. Papageorge, E., and Mitchell, G. L., Jr., *J. Nutrition 37*, 531 (1949).
549. Paris, René, *Produits Pharm. 6*, 543, 606 (1951).
550. Paris, René, *Compt. Rend. Acad. Sci. Paris 235*, 1329 (1952).
551. Parrot, J. L., and Gabe, M., *Compt. Rend. Soc. Biol. 141*, 363 (1947).
552. Parsonnet, A. E., Simon, F., and Bernstein, A., *Med. Soc. New Jersey J. 47*, (1950).
553. Parsons, R. J., and McMaster, P. D., *J. Exptl. Med. 68*, 353 (1938).
554. Paterson, J. C., *Arch. Path. 29*, 345 (1940).
555. Paterson, J. C., *Canad. Med. Assoc. J. 44*, 114 (1941).
556. Patt, H. M., Smith, D. E., Tyree, E. B., and Straube, R. L., *Proc. Soc. Exptl. B Med. 73*, 18 (1950).
557. Patt, H. M., Straube, R. L., Tyree, E. B., Swift, M. N., and Smith, D. E., *Am. Physiol. 159*, 269 (1949).
558. Patt, H. M., Swift, M. N., Tyree, E. B., and John, E. S., *Am. J. Physiol. 150*, (1947).
559. Patt, H. M., Swift, M. N., Tyree, E. B., and Straube, R. L., *Science 108*, (1948).
560. Patt, H. M., Tyree, E. B., Straube, R. L., and Smith, D. E., *Science 110*, 2 (1949).
561. Pautrizel, R., Pautrizel-Bézian, A., and Tayeau, F., *J. Physiol., Par. 42*, 7 (1950).
562. Pavlovsky, A., and Castellanos, H., *Rev. Assoc. Méd. Argentina 62*, 309 (1948).
563. Peck, F. B., and Mann, M., *Am. J. Med. Sci. 217*, 277 (1949).
564. Peck, S. M., Rosenthal, N., and Erf, L. A., *Arch. Dermatol. Syphilol. 35*, 8 (1937).
565. Peluse, S., *Arch. Otolaryngology 44*, 668 (1946).
566. Penna, C. de P., *Hospital* (Rio de Janeiro) *43*, 781 (1953).
567. Perkin, A. G., *J. Chem. Soc. 81*, 473 (1902).
568. Perkin, A. G., *J. Chem. Soc. 97*, 1776 (1910).
569. Perkin, A. G., and Everest, A. E., *The Natural Organic Colouring Matters* (Lon mans, Green & Co., London) (1918).
570. Perkin, A. G., and Pate, L., *J. Chem. Soc. 67*, 644 (1895).

571. Perlmann, G. E., Glenn, W. W. L., and Kaufman, D., *J. Clin. Invest. 22*, 627 (1943).
572. Persike, E. C., and Ureen, H. J., *Proc. Soc. Exptl. Biol. Med. 65*, 339 (1947).
573. Phillips, G. W. M., Aceto, N., Eskew, R. K., and Hurley, R., U. S. Dept. Agr., Bur. Agr. Ind. Chem. AIC-264 (Eastern Regional Research Laboratory) March 1950 (Processed).
574. Piersol, G. A., *Human Anatomy, Ed. 7*, 2104, pp., Lippincott, Phila. (1919).
575. Plouvier, V., *Compt. Rend. Acad. Sci. Paris 216*, 459 (1943).
576. Plungian, M. B., U. S. Pat. No. 2,451,772 (1948).
577. Plungian, M. B., Munch, J. C., and Wolffe, J. B., *J. Pharm. Exptl. Therap. 93*, 383 (1948).
578. Porter, W. L., Brice, B. A., Copley, M. J., and Couch, J. F., U. S. Dept. Agr., Bur. Agr. Ind. Chem. AIC-159 (Eastern Regional Research Laboratory) July 1947 (Processed).
579. Porter, W. L., Dickel, D. F., and Couch, J. F., *Arch. Biochem. 21*, 273 (1949).
580. Porter, W. L., and Fenske, C. S., Jr., *J. Assoc. Official Agr. Chem. 32*, 714 (1949).
581. Power, F. B., and Salway, A. H., *J. Chem. Soc. 105*, 767 (1914).
582. Preston, R. K., Avakian, S., Beiler, J. M., Moss, J. N., and Martin, G. J., *Exp. M. & S. Basel 11*, 1 (1953).
583. Quevauviller, A., and Chabrier, P., *J. Physiol., Par. 44*, 601 (1952).
583a. Quick, A. J., *Am. J. Clin. Path. 19*, 1016 (1949).
584. Quick, A. J., *The Physiology and Pathology of Hemostasis*, 188 pp., Philadelphia, Pa. (Lea and Febiger) (1951).
585. Quick, A. J., *J. Am. Med. Assoc. 145*, 4 (1951).
586. Quick, A. J., Shanberge, J. N., and Stefanini, M., *Am. J. Med. Sci. 217*, 198 (1949).
587. Quick, A. J., Shanberge, J. N., and Stefanini, M., *J. Lab. Clin. Med. 34*, 761 (1949).
588. Quick, A. J., and Stefanini, M., *J. Lab. Clin. Med. 33*, 819 (1948).
589. Quick, A. J., and Stefanini, M., *J. Lab. Clin. Med. 34*, 973 (1949).
590. Quick, A. J., and Stefanini, M., *J. Lab. Clin. Med. 34*, 1203 (1949).
591. Quick, A. J., and Stefanini, M., *Am. J. Physiol. 160*, 572 (1950).
592. Rabaté, J., *Bull. Soc. Chim. Biol. 12*, 974 (1930).
593. Rae, J., *Pharm. J. 109*, 111 (1949).
594. Raiman, R. J., Later, E. R., and Necheles, H., *Science 106*, 368 (1947).
595. Raiman, R. J., and Necheles, H., *Proc. Soc. Exptl. Biol. Med. 66*, 431 (1947).
596. Randall, L. O., and Sevringhaus, E. L., *Arch. Biochem. 22*, 132 (1949).
597. Randoin, L., and Lecoq, R., *Bull. Soc. Chim. Biol. 9*, 513 (1927).
598. Rapoport, S., *Proc. Soc. Exptl. Biol. Med. 64*, 478 (1947).
599. Rayle, A., and Papageorge, E., Am. Chem. Soc. Abstracts of Papers, 113th Mtg., Chicago, p. 20-C (April 1948).
600. Reinhardt, W. O., *Proc. Soc. Exptl. Biol. Med. 58*, 123 (1945).
601. Reinhardt, W. O., and Bloom, B., *Proc. Soc. Exptl. Biol. Med. 72*, 551 (1949).
602. Reinhardt, W. O., and Li, C. H., *Proc. Soc. Exptl. Biol. Med. 58*, 321 (1945).
603. Rekers, P. E., Coulter, M. P., and Warren, S. L., *Arch. Surg. 60*, 635 (1950).
604. Rekers, P. E., and Field, J. B., *Science 107*, 16 (1948).
605. Rekers, P. E., and Marti, N. F., *Am. J. Med. Sci. 221*, 191 (1951).
606. Reppert, E., Donegan, J., and Hines, L. E., *Proc. Soc. Exptl. Biol. Med. 77*, 318 (1951).
607. Richards, R. K., *J. Lab. Clin. Med. 33*, 1464 (1948).
608. Richards, R. K., and Kueter, K. E., *J. Pharmacol. Exptl. Therap. 94*, 372 (1948).
609. Rigdon, R. H., *Arch. Surg. 41*, 101 (1940).
610. Rigdon, R. H., *Surgery 8*, 839 (1940).
611. Roberts, E., Griffith, J. Q., Jr., and Kimbrough, R. A., *Am. J. Obst. Gyn. 47*, (1944).
612. Roberts, L. J., Blair, R., and Bailey, M., *J. Pediat. 11*, 626 (1937).
613. Robertson, C., Lash, J., Cornbleet, T., and Grossman, M. I., *Proc. Soc. Ex Biol. Med. 77*, 164 (1951).
614. Rochleder, Fr., and Hlasiwetz, H., *Ann. Chem. Pharm. 82*, 197 (1852).
615. Rodriguez, R., and Root, H. F., *New Engl. J. Med. 238*, 391 (1948).
616. Rodwell, C. N., *Nature 165*, 773 (1950).
617. Rosa, L., and Cenciotti, L., *Arch. sci. med. 89*, 467 (1950); *Chem. Abs. 45*, 746
618. Rosenfeld, R. E., and Tuft, H. S., *Am. J. Clin. Path. 17*, 405 (1947).
619. Rosenthal, R. L., *Science 110*, 43 (1949).
620. Rosenthal, R. L., *J. Lab. Clin. Med. 34*, 1321 (1949).
621. Roskam, J., Renard, Ch., and Swalüe, L., *Blood 3*, 1112 (1948).
622. Rossi, R., and Lottini, A., *Riv. Clin. pediat. 50*, 627 (1952).
623. Rossman, P. L., *Ann. Internal Med. 14*, 281 (1940).
624. Roth, L. W., and Shepperd, I. M., *Science 108*, 410 (1948).
625. Rudy, A., Beaser, S. B., and Seligman, A. M., *Arch. Internal Med. 73*, 23 (194
626. Rumball, J. M., *Military Surgeon 105*, 53 (1949).
627. Rusznyák, St., and Benkó, A., *Science 94*, 25 (1941).
628. Rusznyák, St., and Szent-Györgyi, A., *Nature 138*, 27 (1936).
629. Rutherford, C. W., *J. Indiana State Med. Assoc. 41*, 494 (1948).
630. Sando, C. E., and Bartlett, H. H., *J. Biol. Chem. 41*, 495 (1920).
631. Sando, C. E., and Lloyd, J. U., *J. Biol. Chem. 58*, 737 (1924).
632. Sando, W. J., *J. Heredity 30*, 271 (1939).
633. Sanford, H. N., Butler, S., and Kennedy, S. R., Jr., *Am. J. Diseases Children 7* 609 (1948).
634. Sannié, C., and Dussy, J., *Compt. Rend. Acad. Sci. Paris 222*, 918 (1946).
635. Sauramo, H., *Ann. chir. gyn. fenn. 41*, 264 (1952).
636. Scarborough, H., *Lancet* 1940, II, 644.
637. Scarborough, H., *Biochem. J. 39*, 271 (1945).
638. Scarborough, H., and Bacharach, A. L., *Vitamins & Hormones 7*, 1, New Yor Academic Press (1949).
639. Scarborough, H., and Stewart, C. P., *Lancet* 1938, II, 610.
640. Schaefer, W., *Z. ges. exp. Med. 108*, 725 (1941).
641. Schaffenburg, C., Masson, G. M. C., and Corcoran, A. C., *Proc. Soc. Exptl. Bio Med. 74*, 358 (1950).
642. Schiller, A. A., *Am. J. Physiol. 165*, 293 (1951).
643. Schmidt, E., *Apoth.-Ztg. 16*, 357 (from *Chem. Zentr.* 1901, II, 121).
644. Schmidt, E., *Arch. Pharm. 242*, 210 (1904).
645. Schmidt, E., *Arch. Pharm. 246*, 214 (1908).
646. Schmidt, Von H., Marx, R., and Festl, B., *Dtsch. med. Wschr. 75*, 790 (1950).
647. Schnir, M., *Revista Argentina de Reumatologia* (Buenos Aires) *15*, 232 (1951 Abs. in *J.A.M.A. 146*, 972 (1951).
648. Schoenkerman, B. B., and Justice, R. S., *Ann. Allergy 10*, 138 (1952).
649. Schraufstätter, E., *Experientia 4*, 484 (1948).
650. Schraufstätter, E., and Deutsch, S., *Z. Naturforsch. 3b*, 163 (1948).
651. Schraufstätter, E., and Deutsch, S., *Z. Naturforsch. 3b*, 430 (1948).
652. Schraufstätter, E., and Deutsch, S., *Z. Naturforsch. 4b*, 276 (1949).
653. Schreiber, M., and Elvehjem, C. A., *J. Nutrition 54*, 257 (1954).
654. Schrek, R., *Endocrinology 45*, 317 (1949).

655. Schultzer, P., *Lancet* 1933, II, 589.
656. Schultzer, P., *Acta Med. Scand. 88*, 317 (1936).
657. Schunck, E., *Chem. Gaz. 17*, 201 (1859).
658. Schunck, E., *Lit. Phil. Soc. Manchester Mem. Series 2, 15*, 122 (1860).
659. Schunck, E., *Chem. News 57*, 60 (1888).
660. Schunck, E., *J. Chem. Soc. 53*, 262 (1888).
661. Schunck, E., *J. Chem. Soc. 67*, 30 (1895).
662. Schuster, N. H., *J. Path. Bact. 44*, 29 (1937).
663. Schwartz, S. O., and Armstrong, B. E., *New Engl. J. Med. 239*, 431 (1948).
664. Schweier, P., *Klin. Wochenschr. 29*, 328 (1951).
665. Schweppe, J. S., Lindberg, H. A., and Barker, M. H., *Am. Heart J. 35*, 393 (1948) (correction *35*, 824 (1948)).
666. Scuderi, G., *Ann. Ottalmol. e clin. oculist. 76*, 275 (1950), *Chem. Abs. 46*, 2645.
667. Seegers, W. H., *Circulation 1*, 2 (1950).
668. Seifter, J., *Bull. schweiz. Akad. Med. Wiss. Special Edition of 8*, No. 1/2, 67 (1952). Symposium on the influence of the hypophysis and the adrenal cortex on biological reactions, Zurich, September, 1951.
669. Seifter, J., Baeder, D. H., and Begany, A. J., *Proc. Soc. Exptl. Biol. Med. 72*, 277 (1949).
670. Seifter, J., Baeder, D. H., and Dervinis, A., *Proc. Soc. Exptl. Biol. Med. 72*, 136 (1949).
671. Selye, H., *J. Clin. Endocrinol. 6*, 117 (1946).
672. Semenza, F., *Acta Vitaminol. 3*, 257 (1949); *Chem. Abs. 46*, 11366a.
672a. Serowy, C., *Derm. Wschr. 127*, 590 (1953).
673. Seshadri, T. R., *J. Ind. Chem. Soc., Ind. & News Ed. 11*, 35 (1948).
674. Sevestre, J., Fabianek, J., Neumann, J., and Lavollay, J., *Bull. soc. chim. biol. 33*, 1571 (1951).
675. Sevestre, J., Fabianek, J., Neumann, J., and Lavollay, J., *Bull. soc. chim. biol. 34*, 135 (1952).
676. Sévin, A., *Compt. Rend. Acad. Sci. Paris 216*, 505 (1943).
677. Shanno, R. L., *Am. J. Med. Sci. 211*, 539 (1946).
678. Shanno, R. L., Griffith, J. Q., Jr., and LaMotte, W. O., Jr., *Am. J. Ophthalmology 30*, 1556 (1947).
679. Shapiro, S., and Spitzer, J. M., *Angiology 5*, 64 (1954).
680. Shibata, K., and Shimokoriyama, M., *J. Chem. Soc. Japan* (Pure Chem. Sect.) *20*, 36 (1949); *Chem. Abs. 45*, 2939b.
681. Shimizu, M., *et al.*, Jap. Patent No. 7547 ('51) Dec. 4; *Chem. Abs. 47*, 5079h.
682. Shimizu, M., and Kirisawa, M., Jap. Patent No. 7247 ('51) Nov. 20; *Chem. Abs. 47*, 5079g.
683. Shimizu, M., Kirisawa, M., and Ogawa, R., *J. Pharm. Soc. Japan 71*, 875 (1951); *Chem. Abs. 46*, 4004a.
684. Shlevin, E. L., and Lederer, M., *Ann. Internal Med. 21*, 332 (1944).
685. Shumacker, H. B., Jr., *Angiology 2*, 476 (1951).
686. Shumacker, H. B., Jr., Radigan, L. R., Ziperman, H. H., and Hughes, R. R., *Angiology 2*, 100 (1951).
687. Shute, E., *Canad. Med. Assoc. J. 45*, 542 (1941).
688. Shute, E. V., Vogelsang, A. B., Skelton, F. R., and Shute, W. E., *Surg. Gynec. Obst. 86*, 1 (1948).
688a. Sieler, H., *Deut. Gesundhwes. 7*, 469 (1952).
689. Sieve, B. F., *Science 116*, 373 (1952).
690. Siguier, F., Giudicelli, R., Chabrier, P., and Trelat, J., *Sem. Hôp. 25*, 3423 (
691. Simonsberg, H., *Ugeskrift for Laeger, Copenhagen, 114*, 971 (1952).
692. Skelton, F., Shute, E., Skinner, H. G., and Ward, R. A., *Science 103*, 762 (
693. Smirnova-Zamkova, A. I., *Am. Rev. Soviet Med. 3*, 534 (1946).
694. Smith, D. E., Patt, H. M., Tyree, E. B., and Straube, R. L., *Proc. Soc. Biol. Med. 73*, 198 (1950).
695. Smith, H. G., *J. Proc. Roy. Soc. N.S.W. 31*, 177, 377 (1897).
696. Smith, H. G., *J. Chem. Soc. 73*, 697 (1898).
697. Smith, W. W., Smith, F., and Thompson, E. C., *Proc. Soc. Exptl. Biol. Me* 529 (1950).
698. Sokoloff, B. T., Eddy, W. H., and Cone, G., *A.M.A. Arch. Path. 54*, 197 (
699. Sokoloff, B. T., Eddy, W. H., and Redd, J. B., *J. Clin. Invest. 30*, 395 (1951
700. Sokoloff, B. T., and Redd, J. B., *Florida Southern College*, Monograph I, Par pp. (1949).
701. Sokoloff, B. T., and Redd, J. B., *Florida Southern College*, Monograph I, Pa 58 pp. (1949).
702. Sokoloff, B. T., and Redd, J. B., *Florida Southern College*, Monograph I, Par 54 pp. (1949).
703. Sokoloff, B. T., Redd, J. B., and Dutcher, R., *Science 112*, 112 (1950).
704. Sokoloff, B. T., Redd, J. B., and Dutcher, R., *Proc. Soc. Exptl. Biol. Med.* (1950).
705. Sokoray, L., and Czimmer, A. G., *Arch. Exptl. Path. Pharmakol. 190*, 622 (
706. Soloff, L. A., and Bello, C. T., *Am. J. Med. Sci. 215*, 655 (1948); *215*, 660(
707. Sonneck, H. J., *Derm. Wschr. 125*, 313 (1952).
708. Sosa, A., and Plouvier, V., *Compt. Rend. Acad. Sci. Paris 226*, 955 (1948).
709. Soulier, J. P., *Sang 19*, 78 (1948).
710. Spiess, A., and Sostmann, E., *Arch. Pharm. 172*, 75 (1865).
711. Springer, J. P., and Shannon, F. A., *Ariz. Med. 6*, 24 (1949).
712. Staehler, W., Matis, P., and Bauer, K., *Med. Welt 20*, 118 (1951).
713. Stahmann, M. A., Huebner, C. F., and Link, K. P., *J. Biol. Chem. 138*, 513 (
714. Stead, E. A., Jr., and Warren, J. V., *J. Clin. Invest. 23*, 279 (1944).
715. Stead, E. A., Jr., and Warren, J. V., *J. Clin. Invest. 23*, 283 (1944).
716. Stefanini, M., and Crosby, W. H., *Proc. Soc. Exptl. Biol. Med. 73*, 301 (195
717. Stein, W., *J. Prakt. Chem. 58*, 399 (1853).
717a. Steinitz, K., and Turkand, H., *Jour. Clin. Invest. 19*, 285 (1940).
718. Stevens, F. A., Moore, D., and Baer, H., *J. Allergy 22*, 165 (1951).
719. Stevenson, A. E., *Food Res. 15*, 150 (1950).
720. Stocker, F. W., *Arch. Ophthalmology 37*, 583 (1947).
721. Stocker, F. W., *N. Y. State J. Med. 49*, 58 (1949).
722. Stocker, F. W., *Arch. Ophthalmology 41*, 429 (1949).
723. Storer, J. B., and Coon, J. M., *Proc. Soc. Exptl. Biol. Med. 74*, 202 (1950).
724. Storey, R. H., Wish, L., and Furth, J., *Proc. Soc. Exptl. Biol. Med. 74*, 242 (1
725. Straube, R. L., Patt, H. M., Tyree, E. B., and Smith, D. E., *Proc. Soc. Exptl. Med. 71*, 539 (1949).
726. Strean, L. P., *Dental Items of Interest 70*, 153 (1948).
727. Sure, B., and Theis, R. M., *Proc. Soc. Exptl. Biol. Med. 37*, 646 (1938).
728. Suzuki, T., and Mori, Y., *J. Mie Med. Coll. 2*, 175 (1951); *Chem. Abs. 47*, 4
729. Swann, R. V., *J. Pharm. Pharmacol. 1*, 323 (1949).
730. Swayne, V. R., Beiler, J. M., and Martin, G. J., *Proc. Soc. Exp. Biol. & Me* 384 (1952).